COMMUNITY HEALTH CELL
I Block
Bangalore-560034
India

CASSAVA, CYANIDE, AND EPIDEMIC SPASTIC PARAPARESIS

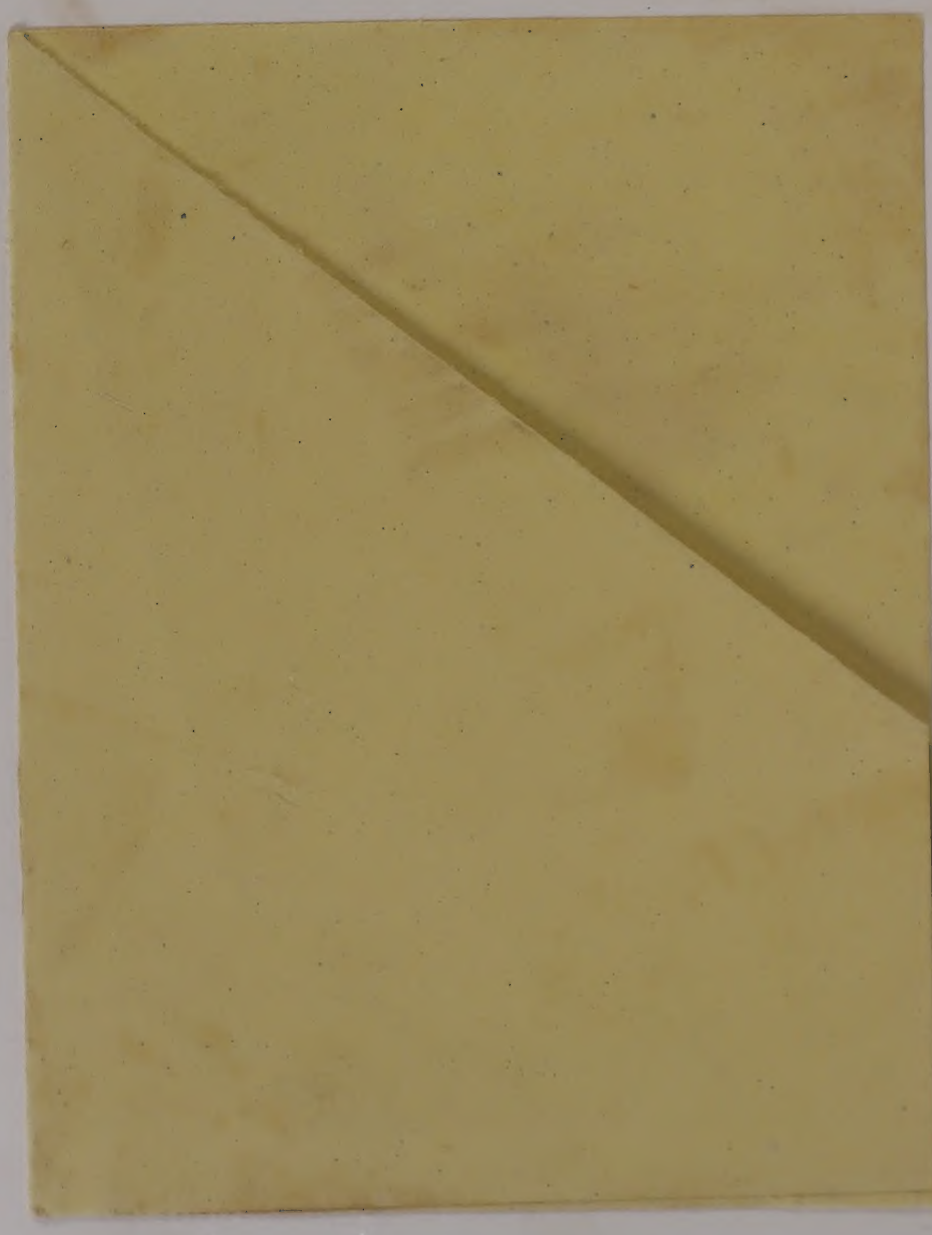
A study in Mozambique on dietary cyanide exposure

by

Hans Rosling

Uppsala 1986

890
890



ACTA UNIVERSITATIS UPSALIENSIS
Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine

19

COMMUNITY HEALTH CELL
~~47/1~~ St. ~~Mark's Road,~~ Bangalore – 560 ~~001~~
THIS BOOK MUST BE RETURNED BY

CASSAVA, CYANIDE, AND EPIDEMIC SPASTIC PARAPARESIS

A study in Mozambique on dietary cyanide exposure

by

Hans Rosling

Uppsala 1986

ACTA UNIVERSITATIS UPSALIENSIS
Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine

19

CASSAVA, CYANIDE, AND EPIDEMIC SPASTIC PARAPARESIS

A study in Mozambique on dietary cyanide exposure

by

Hans Rosling

Uppsala 1986

Doctorial thesis at Uppsala University, 1986.
ISBN 91-554-1798-1
ISSN 0282-7476

ABSTRACT

Rosling, H 1986. Cassava, cyanide, and epidemic spastic paraparesis. A study in Mozambique on dietary cyanide exposure. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine, 19. 52 pp. Uppsala. ISBN 91-554-1798-1

An epidemic of 1102 cases of spastic paraparesis in a drought-stricken area of Mozambique was associated with a high dietary intake of cyanide from cassava roots. Cassava was the only crop to survive the drought. Due to lack of other food the roots, which had a high cyanide content, were eaten without the prolonged sun-drying normally applied to reduce toxicity. High cyanide intake was indicated by very high serum levels of thiocyanate, the detoxification product of cyanide. Follow-up studies showed that the population affected also had a low intake of sulphur, measured as the urinary excretion of inorganic sulphate. The combination of high cyanide and low sulphur intake is the most probable etiology of the paraparesis observed but the results are not conclusive. The neurological lesions may have been caused by impaired synthesis of essential sulphur compounds or they may have been an effect of cyanide per se.

The instability of cyanide in blood precluded its determination. However, the addition of silver ions to blood specimens was found to stabilize cyanide. This new method will facilitate transport of specimens in tropical climates and thereby future determinations of blood cyanide in cassava-eaters.

The ankle clonus rate was high among apparently healthy children from the affected area. Children with clonus had higher cyanide intake, measured as urinary thiocyanate. Further studies indicated that the thyroid gland, in the presence of an adequate iodine intake, can adapt to a heavy body burden of thiocyanate without development of goitre or hypothyroidism.

Cassava toxicity may cause problems under special circumstances. However, the yield of cassava is high and an increased cultivation may thereby in many areas improve the health of poor peasant families, provided that efficient methods for cyanide reduction are used, that some protein-rich supplementary food is available, and that iodine supplementation is given when necessary.

Key words: Cassava, cyanide, epidemic, spastic paraparesis, sulphur, inorganic sulphate, thiocyanate, ankle clonus, thyroid function.

Hans Rosling, Department of Internal Medicine and International Child Health Unit, University Hospital, S-751 85 Uppsala; and Department of Clinical Chemistry, Linköping University, Sweden.

© Hans Rosling 1986
Printed in Sweden by
Tryckkontakt, Uppsala.

COMMUNITY HEALTH CELL
~~47/1~~. (~~First Floor~~) St. ~~Marks Road~~,
Bangalore - 560 001.
DR-415
890

"Särdeles bör han vara uppmärksam på beskaffenheten av allmogens dryck och födoämnen, när däraf, vid inträffad missväxt, otjenlig väderlek vid bergningstiden, eller andra tillfälligheter, farosoter eller skada kunna befaras".

8 § Tjänsteinstruktion för Sveriges provinsialläkare, 1822.

"He should be particularly observant of the quality of the peoples' beverage and food, when due to failure of crops, unsuitable weather during harvest, or other circumstances, epidemics or harm may be feared".

§ 8 Instructions for Swedish general practitioners, 1822.

This dissertation is based on the following papers, which are referred to by their Roman numerals:

I. Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 1. Epidemiology and clinical and laboratory findings in patients.
Ministry of Health, Mozambique, text by:
Cliff J, Martelli A, Molin A & Rosling H.
Bull WHO 1984; 62: 477-84.

II. Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 2. Nutritional factors and hydrocyanic content of cassava products.
Ministry of Health,Mozambique, text by:
Casadei E, Jansen P, Molin A, Rodrigues A & Rosling H.
Bull WHO 1984; 62: 485-92.

III. Association of high cyanide and low sulphur intake in cassava-induced spastic paraparesis.
Cliff J, Lundquist P, Mårtensson J, Rosling H & Sörbo B.
Lancet 1985; ii: 1211-13.

IV. Determination of cyanide in whole blood, erythrocytes, and plasma.
Lundquist P, Rosling H & Sörbo B.
Clin. Chem. 1985; 31: 591-95.

V. Ankle clonus correlating with cyanide intake from cassava in rural children from Mozambique.
Cliff J, Essers S & Rosling H.
(Submitted for publication)

VI. Thyroid function in a cassava-eating population affected by epidemic spastic paraparesis.
Cliff J, Lundqvist P, Rosling H, Sörbo B & Wide L.
(Submitted for publication)

Reprints were made with the permission of the journals.

CONTENTS

Abbreviations.....6

Introduction.....7

Cassava toxicity.....8

Cyanide metabolism.....10

Tropical myelopathy syndromes.....12

Aims of the present study.....15

Study population and methods.....16

Results.....22

Discussion.....32

General summary.....41

Acknowledgements.....42

References.....44

ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
CSF	Cerebrospinal fluid
EC	Enzyme committee
HCN	Hydrogen cyanide
HTLV-I	Human T-lymphotropic virus type-I
LDC	Least developed countries
SCN	Thiocyanate
SEM	Standard error of the mean
T3	Triiodothyronine
T4	Thyroxine
T3U	T3 uptake test
TSH	Thyroid stimulating hormone

The present study on dietary cyanide exposure from cassava was initiated while the author was working as District Medical Officer in Nacala-Porto, a seaport in northern Mozambique. The work included both the management of health care and, in cooperation with a colleague, provision of the second level of medical care for the 360,000 inhabitants of the districts of Maiaia, Nacala, and Memba. The health policy was fully adequate (Ministério da Saúde 1980) but endeavours to promote health were crippled by scarcity of resources and lack of trained personnel. The medical resources available to the population were in fact less than 1% of those available in industrialized countries (Figure 1). The majority of the population lived in rural areas, and their disease pattern was

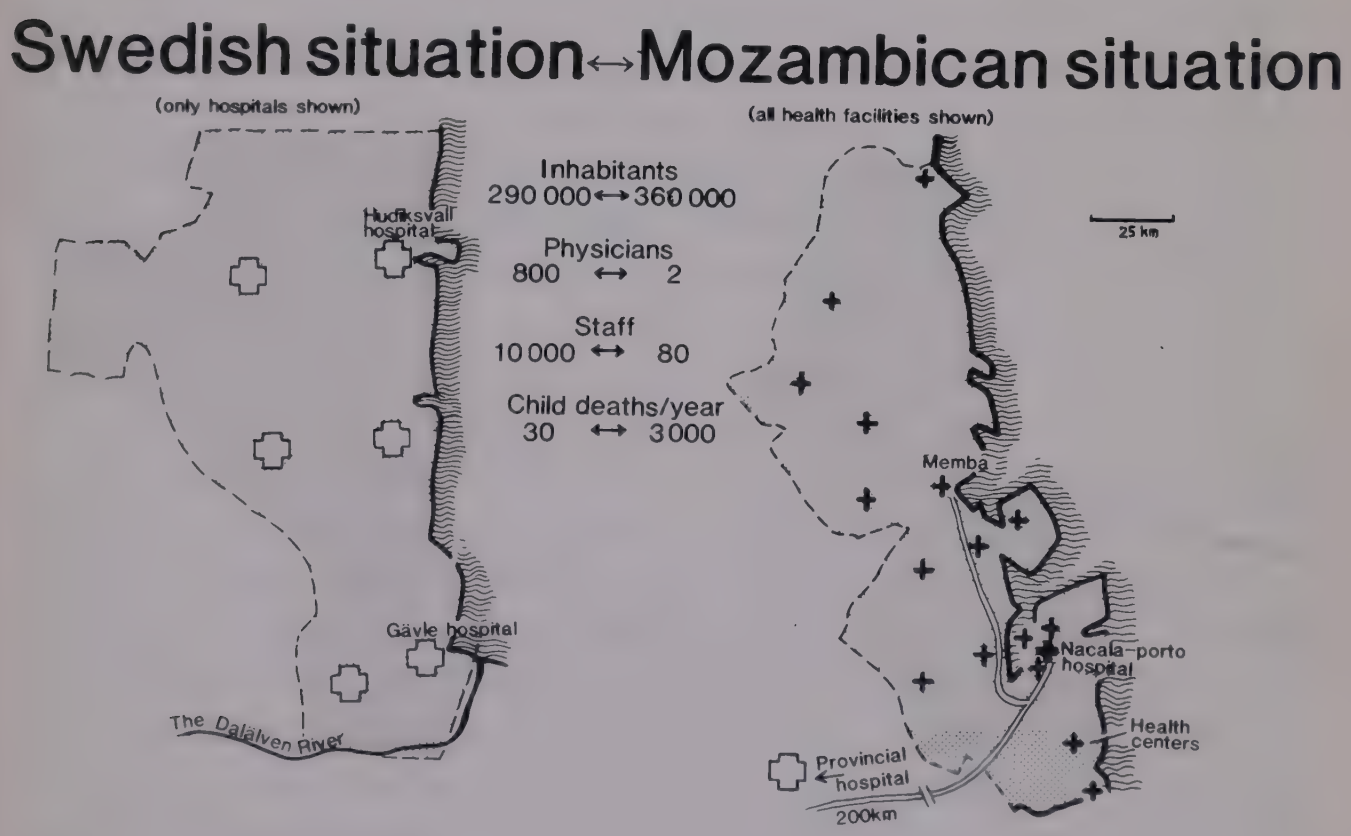


Figure 1. Comparison of medical resources and child mortality in the catchment area of Gävle hospital in Sweden and Nacala-Porto Hospital in Mozambique.

dominated by infectious diseases and malnutrition (UNICEF 1984) with such underlying causes as inadequate supply of water and sanitation, high illiteracy rate, low agricultural productivity, and stagnation of the world economy.

An additional problem arose in 1981 when the rural area surrounding Nacala-Porto suffered a severe drought. In August of that year a remote health post in the most drought-stricken area reported the sudden appearance of many cases of spastic paraparesis, and the study of these cases indicated an extensive epidemic. An urgent investigation was done in cooperation between local medical staff and visiting specialists from the Ministry of Health with support from the local authorities and population. The results showed an association between paraparesis and a high dietary cyanide exposure from insufficiently detoxified cassava roots, which were the staple food in the area.

The present thesis summarizes studies on the epidemic in 1981, follow-up studies on the dietary cyanide exposure of the affected population in 1982-83 and studies on determination methods for blood cyanide. Further follow-up was rendered impossible in 1984, as the war situation imposed on Mozambique reached this part of the country.

Cassava toxicity

Cassava (Manihot esculenta Crantz) is a perennial 1-2 meter high shrub grown throughout the lowland tropics (see figure on cover). Thanks to its high yields of starchy roots, it is one of the most important staple crops in the tropical world (Cock 1982). Propagation is done by planting stem cuttings from a recently harvested plant. The roots of the new plant can be harvested 12 months later or left in the ground for years without deterioration. However, harvested roots will deteriorate rapidly if they are not processed in order to reduce the water content (Coursey 1973).

Cassava has often been depreciated as a food due to its low protein content and its bulky character (Cutting 1978), which make it difficult to use in the preparation of adequate weaning foods (Cameron & Hofvander 1983). Furthermore, the ability of cassava to grow on poor soils has led to the misconception that it depletes soils more than other crops (Cock 1982). It should be observed, however, that cassava has been estimated to supply about 40% of the caloric requirement in tropical Africa (Okigbo 1980) and its wide use is obviously due to the advantages this crop offers to peasants with limited resources. Compared to alternative staple crops, cassava has a higher caloric yield per unit area as well as per labour input, and it is also more resistant to drought and pests. It allows flexibility in planting and harvesting time and no edible part is needed for propagation, an advantage in famine-threatened areas. The high content of protein, iron, and vitamins A and C in the leaves may compensate for the low content of these nutrients in the roots (Lancaster & Brooks 1983). Roots and leaves of cassava, combined with groundnuts, legumes, and fish will provide a balanced diet (McDowell 1976). Recent attention to the complexity of the tropical farming systems has emphasized that nutritional hazards of cassava must be seen in relation to its advantages (Okigbo 1980).

Dietary cyanide exposure constitutes one of those hazards. Roots and leaves of all cassava varieties contain cyanogenic glycosides, mainly linamarin, as well as hydrolytic enzymes capable of liberating cyanide from the glycosides (Nartey 1981). Any process that ruptures the cell compartments will bring the glycoside in contact with the enzyme and thereby yield hydrocyanic acid (HCN). Cyanogenic glycosides are found in amounts corresponding to 15-400 mg HCN/kg fresh weight (Coursey 1973). The bitterness of cassava roots is related to their cyanide content and cassava varieties are often referred to as bitter or sweet. There is, however, a continuous variation in cyanide content among different varieties, and environmental factors, such as drought, can also increase

cyanide levels (De Bruijn 1973). Higher yielding varieties tend to have higher cyanide content (Thampan 1979).

Since the domestication of cassava in tropical America many thousands of years ago (Jones 1959), man has developed a wide variety of processing methods that reduce the cyanide content and make the roots storable (Coursey 1973). These methods are based on soaking, roasting, or sun-drying. Sun-drying saves labour, but even if applied for months, it is still the least effective way to reduce the cyanide content (Bourdoux et al. 1982). Knowledge about cyanide content of cassava products, especially of prepared meals, is limited, due to difficulties connected with determination of cyanide and cyanogenic compounds. It is not known in what form the cyanide intake occurs but laboratory experiments suggest that it is mainly consumed as intact glycoside (Cooke & Coursey 1981). The cyanide exposure will thus depend on the degree of linamarin breakdown in the body (Barrett et al. 1977; Philbrick et al. 1977).

That cassava consumption does result in cyanide exposure has, however, been clearly shown (Osuntokun 1981; Bourdoux et al. 1978), and acute intoxication has also been described (Cheok 1978). Several disorders observed in cassava-eating populations have been attributed to the effects of continuous exposure to dietary cyanide: malnutrition diabetes (McMillan et al. 1980), congenital malformations (Singh 1981), malignancy (Boyland 1974) and neurological disorders (Wilson 1983). The thiocyanate load resulting from the endogenous conversion of cyanide has been reported as an etiological factor in endemic goitre and cretinism among cassava-eaters (Delange et al. 1983).

Cyanide metabolism

Cyanide exposure in man has been reported from other cyanogenic glycosides than those in cassava. Acute intoxication, sometimes fatal, has been caused by amygdaline, which occurs in apricot kernels (Rubino et al. 1979), bitter almond (Shragg

et al. 1981), and in the quack drug leatrile (Moertel et al. 1981; 1982) used in the United States. The use of linseed as a laxative results in cyanide exposure (Schultz et al. 1983) from various types of glycosides in the seeds (Brimer et al. 1983). Cyanide exposure also occurs from several other sources, such as the antihypertensive drug sodium nitroprusside (Schulz 1984; Vesey & Cole 1985), gases of fires (Symington et al. 1978), tobacco smoke (Brunneman et al. 1977), and occupational exposure in metal industries (Labianca 1979). The use of cyanide for suicide and homicide is wellknown (Winek et al. 1978).

The lethal effects of cyanide result from impairment of the mitochondrial respiratory chain by inhibition of the mitochondrial enzyme, cytochrome oxidase. Cyanide is rapidly absorbed from the gut, but in spite of its high toxicity and prevalence in the human environment, deaths and diseases attributed to cyanide exposure are relatively rare. This can be partly explained by awareness of cyanide toxicity but also by the ability of organisms to trap cyanide in the erythrocyte fraction of the blood and later convert it to the less toxic thiocyanate (Frankenberg & Sörbo 1975). This conversion to thiocyanate occurs intracellularly and requires sulphur, which is provided by enzymatic pathways originating from the dietary sulphur aminoacids (Sörbo 1975). The conversion of cyanide to thiocyanate is normally attributed to a reaction with thiosulphate catalysed by the enzyme rhodanese (EC 2.8.1.1), but another enzyme, mercaptopyruvate sulphurtransferase (EC 2.8.1.2) can also catalyse the conversion of cyanide to thiocyanate (Sörbo 1975).

Intravenous administration of thiosulphate is known to increase the capacity for detoxification of cyanide (Chen et al. 1952). Reduced intake of sulphur aminoacids has consequently been believed to reduce detoxification capacity (Wilson 1983), and continuous cyanide exposure may cause harm by depleting the body of sulphur compounds. Small amounts of

cyanide are oxidized to cyanate and then transformed to carbon dioxide and formate (Boxer & Richards 1952). Cyanide can also convert hydroxocobalamin to cyanocobalamin (Wilson et al. 1971) and a reaction between cyanide and cysteine has been reported to yield 2-iminothiazolidine-4-carboxylic acid (Wood & Cooley 1956). The main part of a cyanide load will however be excreted in the urine as thiocyanate. As thiocyanate is more stable and easier to determine than cyanide, it has been used as an indicator of cyanide exposure. Thiocyanate is far less toxic than cyanide, but since the serum half life is more than 3 days, accumulation can thus occur (Schulz et al. 1979). The thiocyanate ion acts as a pseudohalogen and may interfere with the iodine accumulation of the thyroid (Green 1978; Ermans et al. 1980). Endogenous conversion of dietary cyanide to thiocyanate has been reported as a contributory factor to endemic goitre in cassava-eating populations (Ekpechi 1966; Delange et al. 1982) and also in populations on other diets (Eltom et al. 1985).

Tropical myelopathy syndromes

Neurological syndromes of obscure origin, dominated by signs of myelopathy, have been reported from many populations in tropical countries (Spillane 1973). As these populations have suffered from different forms and degrees of dietary deficiency, such myelopathy syndromes have been regarded as nutritional neuropathies (Cruickshank 1976). This term includes disorders affecting different parts of the nervous system, in which dietary factors are regarded as the most probable etiology. The mechanisms involved are poorly understood, however. During World War II, nutritional neuropathies were much more prevalant among prisoners-of-war in tropical countries than among victims of malnutrition in the European concentration camps (Cruickshank 1976). This suggests that complex disturbances, due to combined effects of deficiencies, toxins, and possibly tropical diseases may cause the neurological lesions, and this group of diseases most probably comprise different etiopathological syndromes. The

nutritional neuropathies have been grouped according to which parts of the nervous system are affected (Cruickshank 1976). However, lesions at different levels often coexist. Peripheral neuropathy, lesions to the cortico-spinal tracts and dorsal columns in the spinal cord, and optic atrophy are the most common findings. The proportions between these lesions vary in the different syndromes described. Peripheral neuropathy is thus the main symptom of beriberi, a disease attributed to the low thiamine content in diets dominated by milled rice. Peripheral neuropathies in prisoners-of-war were sometimes combined with optic atrophy, sensorial deafness, and occasionally symptoms of myelopathy, such as ataxia and spastic paraplegia (Denny-Brown 1947; Gill & Bell 1982). Since the first report by Strachan (1897) a similar syndrome has been reported from several populations in the tropics (Ebrahim & Haddock 1964). A myelopathy syndrome with a dominance of lesions to the myelinated tracts resulting in spastic paraparesis or spinal ataxia has been reported from Jamaica (Montgomery et al. 1964), and a syndrome dominated by peripheral neuropathy and spinal ataxia has been extensively studied in Nigeria (Osuntokun 1968a).

Spastic paraplegia with insidious onset and obscure origin has been described from South Africa (Cosnett 1965), India (Mani 1969), and Colombia (Zaninovic et al. 1981; Roman et al. 1985). Nutritional etiology has also been proposed in these conditions. A recent report on an endemic form of spastic paraparesis with insidious onset among adults on Martinique, suggests that human T-lymphotropic virus-I (HTLV-I) may be a contributory factor in the type of paraparesis seen on that island (Gessain 1985).

Lathyrism is a form of spastic paraparesis with acute or subacute onset, caused by excessive consumption of the drought resistant pea, lathyrus sativa (Spencer & Schaumburg 1983). The disease occurs mostly as epidemics in drought-stricken areas where failure of other crops has made a population entirely dependent on lathyrus sativa (Dwivedi & Prassad 1964;

Gebre-ab et al. 1978). Another type of epidemic spastic paraparesis with acute onset has been reported from an area in Zaire where no lathyrus is consumed (Trolli 1939). The outbreaks of paraparesis occurred during periods with severe dietary deficiencies, and dietary cyanide exposure from cassava have been suggested as an etiological factor (Trolli 1938; Nkamany & Kayinge 1982).

Cyanide intake from cassava dominated diets has also been proposed as a contributory factor in other forms of nutritional neuropathy. The anthropologist Mary Kingsley (1893) reported that a population in West Africa attributed blindness to "a diet too exclusively maniocan". Clarc (1935) suggested that cyanide exposure from cassava was the cause of a neurological syndrome called tropical ataxic neuropathy seen in Nigerians. Peripheral neuropathy and lesions to the dorsal columns in the spinal cord dominated the clinical picture and epidemiological studies related the disease to cyanide exposure from cassava. A high cyanide intake in cases of the disease was indicated by increased plasma thiocyanate (Monekosso & Wilson 1966; Osuntokun et al. 1969) and high blood cyanide levels (Osuntokun et al. 1970). Deficiency of sulphur, needed for cyanide detoxification, was also proposed as an etiological factor. This was supported by the finding of low plasma levels of sulphur-containing aminoacids in cases of the disease (Osuntokun et al. 1968b).

AIMS OF THE PRESENT STUDY

The study was initiated by the investigation of an epidemic of spastic paraparesis in a drought-stricken rural area in Mozambique from August to October 1981. An association was found between the disease and a high dietary intake of cyanide from cassava. Follow-up studies on the affected population in 1982 and 1983 were consequently focused on possible effects of dietary cyanide exposure. Most laboratory investigations were performed in Sweden. The aims were to study:

1. The characteristics of the epidemic of spastic paraparesis observed. (I)

2. The etiological role of a high dietary cyanide intake from cassava. (I, II, III, V)

3. The etiological role of a low dietary intake of sulphur aminoacids. (II, III)

4. Whether a method for determination of cyanide in blood, suitable for field surveys, could be developed. (IV)

5. Whether ankle clonus rate in apparently healthy children was related to the cyanide intake from cassava. (V)

6. Whether thyroid function was affected by a high thiocyanate load from cassava. (VI)

STUDY POPULATION AND METHODS

The population affected by the epidemic lived in the north-eastern part of Nampula Province. This area can be divided into three agricultural zones (Figure 3A)running parallel to the coast (Carvalho 1969; FAO/UNDP/MOZ 1980).

-A dry coastal zone, 10 km wide with very limited agricultural capacity. Cassava is the dominating crop but the population also lives on fishing, trade, and work on sisal plantations as well as in the port and industries of Nacala-Porto, a town with 85,000 inhabitants in the southern part of the zone. The other district centres located in this zone are semiurban localities with less than 5,000 inhabitants.
-An intermediate zone, stretching from 10 to 80 km from the coast, with limited agricultural capacity due to low and irregular rainfall. The population are peasants growing mainly cassava but also maize, sorghum and beans. Cashew nuts are the main cash crop. Due to proximity to the coast, with its trading activities, the population density is sometimes high (50/km2) in relation to the limited agricultural suitability.
-A fertile interior zone situated more than 60-80 km from the coast with higher rainfall and better agricultural suitability. Cassava is the staple crop, but a greater proportion of maize and sorghum is grown, while cotton is the dominating cash crop.

The population in the whole area belongs to the Macua ethnic group with an ancient Arab influence in the coastal zone. During the colonial period cassava, groundnuts, cashew nuts, and cotton were introduced to the area. This profoundly changed agricultural practices, though agricultural methods as such were not improved (Braganca & First 1980). The families live in scattered dwellings, but all belong to an administrative system of local communities. Since Independence in 1975 about 10% of the population has joined newly formed

communal villages. Most of these villages have community health workers. Access to other forms of medical care is limited (Figure 1, page 7), but primary school is available to most children.

Investigations during the epidemic

The outbreak of an epidemic of spastic paraparesis was first reported from the remote health post of Cava, Memba District (Figure 3B) on 13 August 1981. An initial one-day study indicated an extensive epidemic in the area and initiated a two-month investigation. Relief operations, such as food distribution, provision of water and rehabilitation of the crippled were done at the same time as the investigation.

A detailed neurological examination, including history, was performed on a group of 53 cases, 28 of whom lived in the communal village Acordo de Lusaka (referred to as "Lusaka") and 25 that were patients at health centres.

An epidemiological survey was done from August to October among all 500,000 inhabitants in the five districts affected by the epidemic. The survey was based on active case detection by more than 1,000 local community leaders. In a series of meetings, the community leaders were asked to register subjects with difficulty in walking. These subjects were then examined by one of 20 specially trained paramedical workers who made home visits, travelling by motorbike, bicycle, or on foot. On standardized forms they registered age, sex, dwelling place, symptoms, and time of onset, as well as the results of examinations of general health, gait, and knee and ankle reflexes. Results were presented daily to the 3-4 physicians, who included or rejected the cases. When a case was doubtful or came from a formerly non-affected community, the physician visited the subject the following day to perform a more extensive examination. The criteria for a case were visible difficulty in walking due to weakness of both legs, with onset since the last rains, as well as exaggerated knee and ankle

reflexes. Locally available demographic data from the National Census in August 1980 were used to calculate the incidence in each community, which were all mapped during the survey.

In a house-to-house survey of 131 households in Muentaze local community, Maiaia District, all 446 subjects present were examined for paraparesis to study the validity of the epidemiological survey.

A case-control study was done in the village "Lusaka", in the Memba district, selected for its location in the centre of the epidemic. A total of 28 cases were identified after examination of 815 inhabitants. A 24-hour recall on food consumption was done in 17 affected families and in 17 non-affected neighbouring families with age- and sex-matched controls. Blood specimens were obtained from 24 of the cases and from 22 age- and sex-matched neighbour controls.

Nutritional investigation also included collection of information on agricultural practices and dietary habits before and during the drought. This information was obtained by informal interviews and observations during the survey when hundreds of homevisits were made in studied districts. Information was also obtained through public meetings and in interviews with local authorities, teachers, and traders. Systematic interviews on the dietary situation and collection of cassava specimens were done in 10 families in the house-to-house survey, in the 34 families in the case-control study, and finally in 8 families in the Calawane community in the northern part of the affected area. Similar interviews were done in 10 families in the non-affected town Nacala-porto.

Laboratory investigations were done on urine from 13 and CSF from 16 of a group of cases admitted to health centres. A total of 222 blood specimens for thiocyanate determination were obtained from cases at 10 different sites in the affected area, and in 24 cases and 22 controls in the case-control study.

Follow-up investigations in 1982 and 1983

The follow-up investigations were performed during the same season of the year as that in which the 1981 epidemic occurred. The dietary circumstances were similar though not as severe as during the drought year. During the 1982 harvest 27 subjects aged 5-60 years from randomly selected households and 30 schoolchildren were studied in the communal village of "Lusaka". During the 1983 harvest schoolchildren from the affected population were studied in Cava, "Lusaka", and Nakeke (Figure 4). They were compared to cassava-eating children from the non-affected population in the administrative centre of the Memba District, to schoolchildren from the city of Nampula who consumed virtually no cassava, and to Swedish schoolchildren. The number of subjects studied at each site is given in Table I.

Table I Number of subjects studied at each site in the follow-up investigations in 1982-83.

Year	Site	Community	Group	Article III	Article V	Article VI
1982	*"Lusaka"	Rural	General population			27
"	* "	"	Schoolchildren	30		
1983	*"Lusaka"	Rural	Schoolchildren	31	106	106
"	*Cava	"	"		83	83
"	*Nakeke	"	"			87
"	Memba	Semiurban	"	30	134	
"	Nampula	Urban	"	28	52	
"	Sweden	Controls	"	17		

* Indicates sites affected by the epidemic

Nakeke was intended as a non-affected rural site but since some cases were verified in the village, children studied at this site were only included in the thyroid study (VI).

Nutritional information was obtained from peasants, teachers, traders, and health staff in the respective areas. Information on food consumption during the last 24-hour period was obtained from the first 30 schoolchildren in each school in 1983.

Neurological examinations were done on all children examined in 1983, with the child sitting on a table. When testing for clonus, responses in at least one leg of three or more rhythmical contractions of a frequency of about 5 Hz (Hagbarth 1984) were considered positive.

The thyroid glands were examined according to Pérez et al. (1960) on all children studied in 1983. The glands were regarded as enlarged if the palpable volume of the lateral lobes exceeded that of the terminal phalanx of the thumb of the subject examined.

The laboratory investigation was performed on urine specimens from all subjects studied in 1982 and on serum specimens from the subjects in the sample of the general population. In 1983 urine specimens were collected from the first 30 children in each school. Urine was collected with thymol-isopropanol as preservative, and the specimens were frozen within a few hours and then transported frozen to Sweden for analysis. Serum specimens were obtained from the sample of the general population and also transported frozen for analysis.

Analytical methods

Cyanide exposure was measured by determination of thiocyanate, the main detoxication product. Out of the blood specimens collected during the epidemic, 5 frozen sera and plasma from 43 specimens of heparinized blood were determined at Ruchill Hospital, Glasgow, according to Pettigrew & Fell (1972). The remaining heparinized blood specimens, including those from the case-control study were sent via Geneva to the Department of Clinical Chemistry, Linköping University, Sweden and were by then hemolysed. Thiocyanate was determined in the supernatant after centrifugation, using a colorimetric method (Lundquist et al. 1979) after interfering compounds had been eliminated by ion-exchange chromatography on resin Amberlyst A-21 (Lundquist et al. 1983). The same method was used for determinations in serum and urine from the follow-up studies.

Tests for organophosphates, DDT, and Lindane were done on 10 urine specimens at the Hospital for Sick Children, Glasgow. Tests for viruses were done on 13 urine, 16 CSF, and 29 serum specimens collected from the cases during the epidemic. The analyses were performed at the Regional Virus Laboratory, Glasgow. Examination of CSF for cells and for protein by Pandy's test was done in Mozambique. Cyanide content in cassava specimens was determined in the National Food Laboratory in Mozambique using alkaline titration and direct potentiometric methods (Horwitz 1980).

The intake of sulphur aminoacids was assessed as the urinary excretion of total sulphur and inorganic sulphate (Sabry et al. 1965). These compounds as well as ester sulphates were determined by turbidimetric methods (Lundquist et al.1980). The urinary excretion of the various compounds studied was expressed as the creatinine ratio, the latter determined by a routine alkaline picrate method.

Cyanide in blood was determined after stabilization by addition of silver ions. As soon as possible after collection 1 ml of whole blood, 1 ml of erythrocytes or 4 ml of plasma was added to 10 ml of acid silver sulphate reagent and specimens were then stored for various periods at room temperature, at 4 °C, and at -20 °C. After acidification of the sample, cyanide was transferred by aeration into sodium hydroxide and quantified by the König reaction, with sodium hypochlorite as the chlorinating agent.

Serum levels of TSH, T4, and T3 were determined by radio-immuno-assay methods (Odell et al. 1965; Wide 1981). From these values the free T4 index(FT4I) and free T3 index (FT3I) were calculated by multiplication by T3-uptake test, the latter determined by Phadebas T3U Test (Pharmacia, Uppsala, Sweden). Urinary iodine was determined by the catalytic ceric-arsenite method (Sandell et al. 1936) after interfering compounds had been separated on an ion-exchange resin AG 1-X4 according to Sacks (1964).

The disease (I)

Difficulty in walking, mostly with sudden onset, was the main symptom of all 53 of the cases in which a detailed neurological history and examination was done. All cases had symmetrical spastic paraparesis in varying degrees, and 24 of them could not walk without support (Figure 2). All except 4 cases had increased knee and ankle jerk, 31 had sustained ankle clonus, and 28 had increased arm reflexes. Dysarthria was found in 19 cases, diminished visual acuity in 11, and reduced hearing was suspected in some cases. Slight sensory change in the legs was a rare finding. Romberg's sign was absent, and no signs of mental impairment were found. Apart from neurological signs no consistent abnormality was found on general examination. These findings in the 53 cases corresponded to the signs found in hundreds of cases examined during home visits. Cases reexamined one year later showed little improvement as regards neurological signs, but most cases had been trained to walk with crutches.

Figure 2. Children affected by spastic paraparesis.

The epidemic (I)

A total of 1102 cases were identified in the epidemiological survey, and 772 of these came from the Memba district where the disease was first reported. The disease mainly occurred in a rural area of 50 x 100 km, corresponding to the drought-stricken intermediate agricultural zone (Figure 3B). The interior zone was only moderatly affected by drought and hardly at all by the epidemic. The coastal zone suffered a severe drought but had a low incidence of paraparesis. In spite of high incidences in the rural surroundings, not one single case was identified among the 85,000 inhabitants of the town of Nacala-Porto or among the population in the small semi-urban administrative centres of the Nacala and Memba

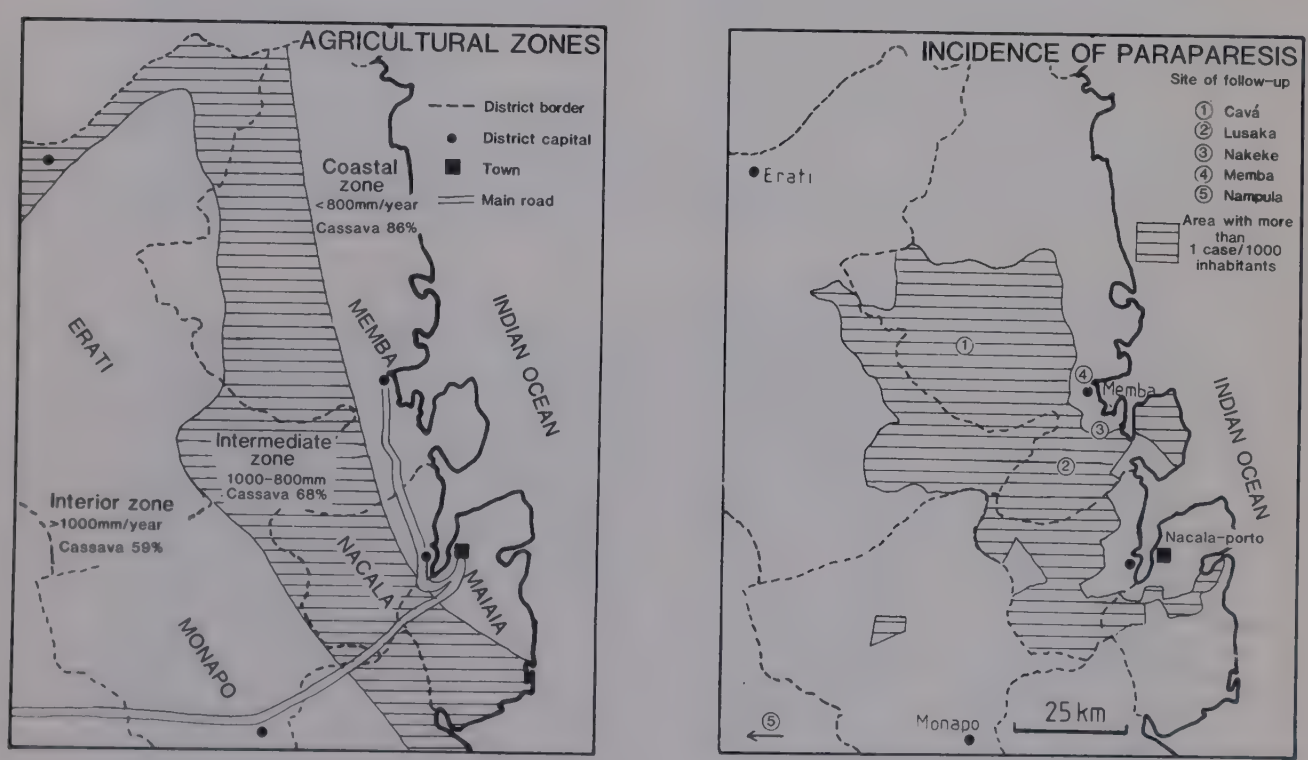


Figure 3A. Agricultural zones, rainfall (FAO/UNDP/MOZ 1980) and % of cultivated land used for cassava (Carvalho 1968) in the five districts studied.

3B. Geographical distribution of the epidemic of spastic paraparesis and sites of follow-up studies.

districts. A special effort was made to obtain valid information on the onset of the cases in the Memba district. The resulting distribution over time, which corresponded to that found in the other districts, is shown in Figure 5. Information on age and sex was obtained in 764 (99%) of the cases recorded in the Memba district; 43% were boys and 22% girls under 15 years of age; 9% were men and 26% women of adult age.

The epidemiological survey was performed independently but with the same methodology in the five affected districts, and similar incidence rates were found in bordering communities on different sides of the district borders. In the house-to-house survey a total of 446 subjects were examined, and of the 9 cases found, 7 had already been identified by the general survey.

The diet (II)

In normal years cassava constituted 80% of the staple food in the affected area, and the information obtained revealed similar agricultural and dietary practices throughout the area. Toxic varieties of cassava dominated the cultivations due to their higher yields, as stated by all peasants interviewed. Cassava was harvested and replanted in August, and the roots were peeled, split and then sun-dried for 3 weeks before storing in the attic for up to 12 months (Figure 4). Roots of toxic varieties were considered safe for consumption only after a minimum of 2 months of drying, and every family reserved some of the previous year's dried cassava to cover the period when newly harvested roots were too toxic for consumption (Figure 5). No other processing method for cassava was known in the area. The dried cassava pieces were used after being pounded into flour, which was then mixed with boiling water to make a thick paste, called "chima". This was eaten with a relish on beans, fish, or vegetables according to availability. Maize and sorghum were eaten during some months following the harvests in February and May respectively.

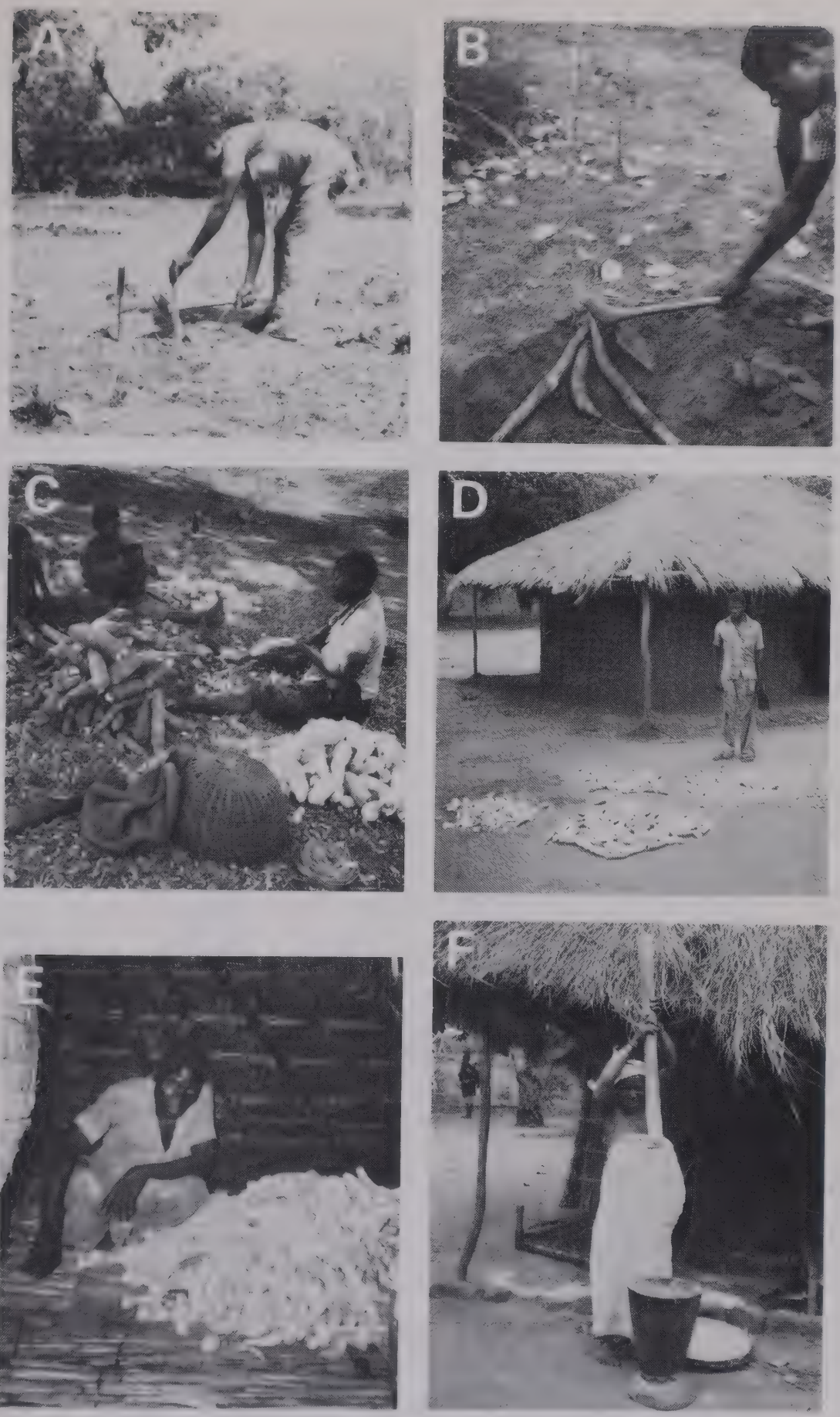


Figure 4. The use of cassava: A. Planting B. Harvesting C. Peeling D. Sun-drying E. Storing F. Pounding

In the drought year 1981 the rainfall was the lowest in memory and record in the area, and the drought was severe in the coastal and intermediate agricultural zones (Figure 3A). All crops except cassava were almost completely lost in these zones, but the more toxic varieties of cassava produced a reasonable harvest in spite of the drought. All peasants interviewed emphasized that the drought had made these roots even more toxic than usual. By the end of June the store of cassava was depleted in most families, and all means of procuring food were exhausted; cassava had to be harvested. The population was unable to detoxify the roots rapidly, and in spite of a more bitter taste than usual the people had no choice but to eat the roots without the prolonged drying normally applied in order to reduce the toxicity (Figure 5). Most families recalled having acute symptoms, such as nausea, vertigo, and confusion, 4-6 hours after ingestion of meals containing these cassava roots. By the end of August these reactions became more rare, which the population attributed to a more sufficient drying of the cassava eaten.

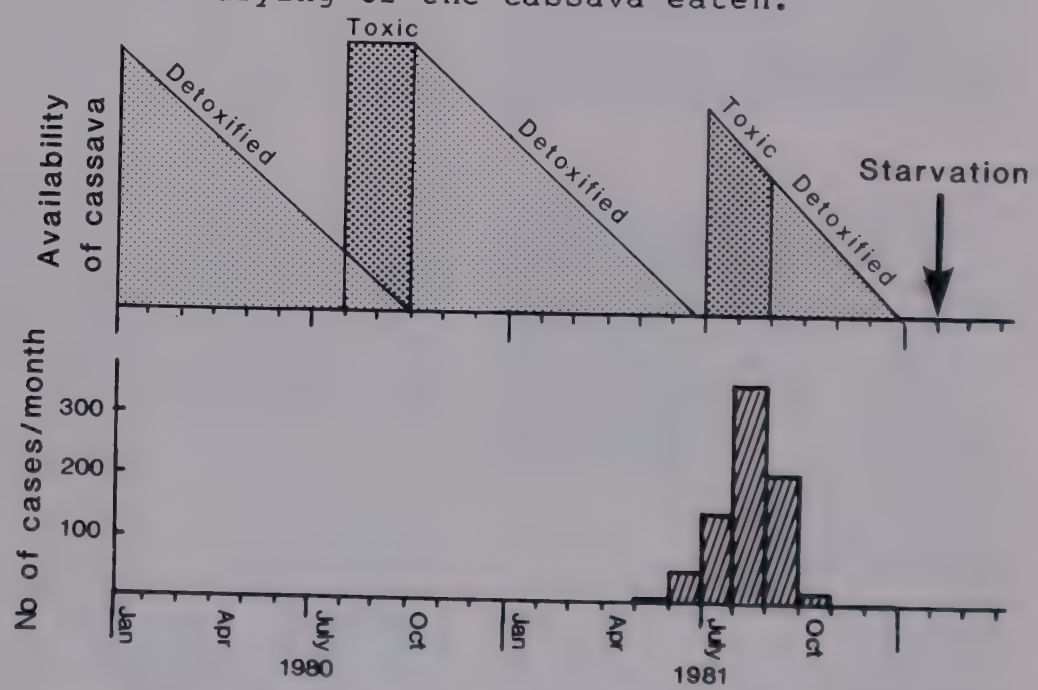


Figure 5. The estimated availability of toxic and detoxified cassava, the period of starvation and the distribution over time of onset of paraparesis in the Memba district.

The case-control study showed that affected as well as non-affected families lived on a diet dominated by cassava and ate

very little protein-rich supplementary food. Nutritional status measured as weight for height did not differ between the members of two groups of families. The families with cases of paraparesis may have had a lower socio-economic status. More of them were dependent on gifts of food, and fewer were members of the consumers' cooperative as compared to non-affected families.The affected families ate more uncooked cassava, whereas the non-affected families ate more meat and fish (Table II). A total of 110 wild plants, eaten during the drought, were collected, and 85 species could be identified, but none of them are reported to cause paraparesis. No specific plant could be identified as having been consumed by all of the cases.

Table II Number of families in the case-control study who ate the various types of food during the last 24-hour period.

	Affected families n=17	Non-affected families n=17	P-value
Cassava, paste	17	17	n.s.
Cassava, uncooked	16	6	<0.001
Cereals	1	1	n.s.
Beans	1	1	n.s.
Fish or meat	0	6	<0.05
Wild plants	1	6	n.s.

n.s. Not significant. P-values calculated by Fisher's exact probability test (two-tail).

Cyanide intake (I,II,III,V,VI)

The mean cyanide content of 15 specimens from fresh roots of the toxic cassava varieties was 327 mg HCN/kg, and the range was 186-446 mg/kg. The corresponding value for 12 pieces dried for 1-8 weeks was 95 (64-178) mg/kg, with small differences related to the drying period. The cyanide intake was estimated by determination of serum and plasma thiocyanate levels; 246 cases with spastic paraparesis had a mean (±SEM) of 329±8 µmol/l. Table III shows the corresponding values of the case and control groups studied in the village of Lusaka during the

drought year, as well as of non-affected subjects from the same village one year after the drought. Serum thiocyanate levels reported from other cassava-eating populations with neuropathy attributed to cyanide exposure are also shown for comparison.

Table III Serum or plasma thiocyanate in neuropathy cases and controls from cassava-eating areas in Mozambique, Nigeria and Tanzania. Mean±SEM in μmol/l.

	Cases	Controls
Mozambique 1981 (drought year)	324±18 (n=24)	288±23 (n=22)
Mozambique 1982 (follow-up study)		250±24 (n=27)
Nigeria (Osuntokun 1981)	127±5 (n=79)	58±2 (n=93)
Tanzania (Makene&Wilson 1972)	93±19 (n=8)	28±8 (n=9)

In the years following the drought the agricultural situation slowly normalised, but food shortages persisted during cassava harvest periods, and the roots were consumed after only 2 and 4 weeks of sun-drying during the 1982 and 1983 harvests respectively. The dietary circumstances during the follow-up studies in 1982 and 1983 cassava harvest periods were thus similar to those during the 1981 drought, though not as severe. Table III shows that subjects from the affected area had high serum thiocyanate levels also during the 1982 harvest, and schoolchildren from the affected area examined at the same time had a urinary thiocyanate excretion of 125±15 mmol/mol of creatinine, which was 30-fold higher than in Swedish controls. During the 1983 harvest urinary thiocyanate was still high in schoolchildren from the same village in the affected area. Table IV shows that these children had higher urinary thiocyanate excretion in comparison with children from a nearby cassava-eating area where no cases of paraparesis had occurred. The thiocyanate excretion in these two groups of

cassava-eating children were about 10-fold higher than those found in non-cassava-eating urban children in Mozambique and Sweden, 8.9±2.7 and 3.4±0.6 mmol/mol creatinine respectively.

Sulphur intake (II,III)

The affected population lost almost their entire production of protein-rich crops during the drought. Due to the low yield of cassava, most families were unable to barter cassava for fish, as they usually did in normal years. A typical answer in the interviews in August 1981 was:

> "-We have cassava roots but not enough time to let them dry before we eat them and there is no other food to eat together with these bitter roots".

This corresponded to the results of the 24-hour recall (Table II), which showed that most families ate virtually no protein-rich supplementary food at the time of the 1981 epidemic. During the 1982 cassava harvest the diet was still cassava-dominated with little protein-rich supplementary food. The intake of sulphur aminoacids was estimated by determination of urinary excretion of inorganic sulphate. In 1982 schoolchildren from the affected area had a mean(±SEM) urinary inorganic sulphate of 0.41±0.04 mol/mol of creatinine while the corresponding value in Swedish schoolchildren was 2.16±0.14. Table IV shows that the excretion of inorganic sulphate and total

Table IV The 1983 urinary excretion of sulphur compounds in cassava-eating children from areas differing with respect to occurrence of spastic paraparesis.

	Affected area n=31	Non-affected area n=30
Thiocyanate	83.9±9.3 *	52.0±12.6
Inorganic sulphate	0.73±0.08*	1.34±0.17
Ester sulphate	0.34±0.03	0.42±0.09
Total sulphur	1.42±0.09*	2.42±0.36

* Significantly different by Student's t-test ($p<0.05$). Results are presented as mean±SEM and given as mol/mol of creatinine, except for thiocyanate which is mmol/mol.

sulphur was significantly lower in children from the area affected by paraparesis in comparison with children from the nearby district centre in the coastal zone, where cassava was a staple food but where no cases of paraparesis had occurred. The excretion of inorganic sulphate in children in the non-affected area was similar to that of urban controls not consuming cassava, and the excretion of ester sulphate was similar in all three groups of Mozambican children studied.

Determination of cyanide in blood (IV)

Determination of blood cyanide levels in the cassava-eating subjects was not possible as the instability of cyanide precluded transport of specimens at the temperature used in the present studies, -4 °C. Laboratory investigations revealed that the addition of silver sulphate to whole blood stabilized cyanide, and after 3 weeks at room temperature, 100 % of the cyanide added was recovered in both whole blood and erythrocytes, whereas only 80% was recovered if no silver ions were added. If silver ions were added to plasma, the recovery immediately after addition rose from 60 to 80 %. The plasma with added silver ions had a constant 80 % recovery for 3 weeks of storing at 4 °C or below, whereas cyanide was successively lost in storage at room temperature, even after addition of silver ions. But of cyanide added to plasma without addition of silver ions, only 15% could be recovered after one day of storage at room temperature. Cyanide vanished in a similar way when added to albumin solutions, which suggests a reaction with albumin or with a substance in the albumin fraction. Linamarin added to plasma gave no detectable cyanide in the assay.

Ankle clonus (V)

The rate of ankle clonus was 22% in apparently healthy school children from the affected population when examined 2 years after the epidemic. The clonus rates among children studied in the two neighbouring non-affected communities were significantly lower, 10% and 6% respectively. Urinary thiocyanate

excretion was also higher in children from the affected area (Table IV), and the 24-hour dietary recall also revealed higher cassava consumption in this group compared to children from non-affected areas. Thiocyanate excretion was determined in 63 of the children from the affected area. The mean (±SEM) in the 12 children with clonus was 125±13 mmol/mol creatinine which was significantly higher ($p<0.001$) compared to the 70±6 mmol/mol of creatinine found in the 51 classmates without clonus.

Thyroid function (VI)

In the follow-up studies, the urinary excretion of thiocyanate in a sample of 27 subjects from the general population in the affected area was 132±28 mmol/mol of creatinine. This was 30-fold higher compared with a Swedish reference group of non-smoking adults. The urinary excretion of iodine determined in 21 of the subjects studied was 79±7.3 µmol/mol of creatinine, which did not differ from a Swedish reference group. The serum levels of TSH and thyroid hormones in the 27 subjects are shown in Table V. Compared to controls, TSH was raised, FT4I reduced, and FT3I increased, resulting in a higher T3/T4 ratio. However, all TSH values were below 10 mU/l, indicating that no one had serious hypothyroidism. The goitre rate in 274 schoolchildren from the study population which were examined the following year was 1.1% (confidence limitse 0.1-4.0% using Poisson distribution).

Table V Serum TSH and T3/T4 ratio in cassava-eating subjects compared to Swedish controls (mean±SEM).

	Cassava-eaters	Controls
TSH mU/l	4.3±0.31 * (n=27)	2.1±0.09 (n=212)
T3/T4 molar ratio	2.9±0.16 * (n=27)	2.0±0.10 (n=27)

* Significant difference with Student's t-test $p<0.001$.

DP 415
890

DISCUSSION

The combination of high cyanide and low sulphur intake from a diet dominated by insufficiently processed cassava with high cyanide content was found to be the most probable etiology of the epidemic of spastic paraparesis. The results are in accordance with earlier reports of possible neurotoxic effects of cyanide exposure from cassava, but a causal relationship could not be established by this retrospective epidemiological study.

The clinical picture, with spastic paraparesis, associated with increased upperlimb reflexes and dysarthria in the most severely affected subjects, indicates a selective lesion of the cortico-spinal tracts. Predominant lesions to one of the most myelinated tracts in the spinal cord in combination with signs of impaired visual acuity and peripheral neuropathy in some cases correspond to the description of nutritional neuropathies of unspecified etiology reported from populations with dietary deficiencies in Zaire (Trolli 1939), Jamaica (Montgomery et al. 1964), India (Mani et al. 1969), and other countries (Cruickshank 1974).

The diet associated with the epidemic was dominated by roots of toxic cassava varieties. The drought itself induced especially high cyanide content in these roots, and on top of that, due to shortage of food, the population had to consume the roots without previous reduction of cyanide, which is normally attained by sun-drying. The cyanide levels found in fresh and dried roots were thus high compared to other reports (Oke 1979; Coursey 1973). In fact an adult's daily intake of 500 g of dried cassava may provide a potential daily load of 50 mg of HCN, which is in the range of the lethal dose of cyanide (Vessey et al. 1976). Intake would be even higher if calculated on the levels in fresh roots, which actually constituted the diet during the epidemic. However, most of the

cyanide content in cassava meals is constituted by intact glycosides (Cooke & Coursey 1981). The total cyanide concentration in cassava specimens was determined in the present study as the sum of free cyanide and cyanide released from cyanogenic compounds. The cyanide load will thus depend on the degree of hydrolysis of ingested glycosides by enzymes in the gut or within the general metabolism (Barrett et al. 1977). That the cyanide load in the study population was close to the lethal range at the time of the epidemic was also indicated by the occurrence of acute toxic reactions after meals and by the very high serum levels of thiocyanate. The thiocyanate levels found in both serum and urine were in fact higher than the levels previously reported from cassava-eating populations (Table III)(Delange 1983), and the main origin of thiocyanate was obviously endogenous conversion of cyanide. The urinary excretion of thiocyanate found in the follow-up study in the affected population one year after the epidemic was still high and corresponds to an endogenous conversion of more than 25 mg of HCN per day in an adult.

The geographical distribution of the disease corresponded well to a certain agricultural zone (Figure 3 A&B) with the highest consumption of toxic cassava. Furthermore, the follow-up studies also verified higher thiocyanate levels in the affected area than in nearby non-affected areas. The ankle clonus rate, measured as a possible indicator of minimal lesions to the cortico-spinal tracts, was higher among apparently healthy children from the population affected by the epidemic compared to a corresponding group of children from non-affected populations. Children with clonus were more exposed to cyanide, as indicated by a significantly higher urinary thiocyanate excretion, which supports the hypothesis that cyanide exposure under certain conditions can be an etiological factor in lesions to the spinal cord.

The appearance of paraparesis corresponded to the period when bitter cassava was consumed without reduction of cyanide

content (Figure 4) and the end of the epidemic coincided with consumption of dried cassava. In the first few months of 1982 a shortage of all forms of cassava occurred, and in those months hunger-oedema and deaths from starvation were observed in the area but no more cases of paraparesis (Cattaneo 1982). A considerable seasonal variation in cyanide intake, measured as urinary thiocyanate levels, has been found in school-children from the affected area, and cyanide intake was highest during the cassava harvest in August-September (Amaral et al. 1985). This association in time between the epidemic and the high cyanide intake is the strongest evidence for an etiological role of cyanide in the observed disease.

Cyanide exposure from insufficiently processed cassava has been suggested as an etiological factor (Trolli 1938; Nkamany 1982) in epidemic spastic paraparesis described in Zaire. The epidemic was first reported from a drought-stricken area where the population consumed cassava (Trolli 1938), and seasonal outbreaks of spastic paraparesis have later been described from the same region (Villén 1948; Lucasse 1952; Anonymous 1982). Cyanide exposure from cassava has also been reported as an etiological factor in nutritional neuropathies observed in cassava-eaters from Nigeria (Monekosso & Wilson 1966, Osuntokun 1981) and Tanzania (Makene & Wilson 1972).
The thiocyanate levels in healthy controls in this study were much higher than those found in the cases of neuropathy among cassava-eaters in Nigeria and Tanzania (Table III). One possible explanation is that cyanide was not an etiological factor. However, the neurological disorders studied in Nigeria and Tanzania were chronic conditions with insidious onset, and cyanide exposure could have been higher in earlier years. Furthermore, cyanide was not suggested as the only etiological factor. Deficiency of sulphur needed for conversion of cyanide to thiocyanate was also suggested as an etiological factor in the neurological disease seen in Nigerian cassava-eaters (Osuntokun et al. 1968b).

Neuropathological changes have been reported in animals after both acute (Lessell 1971) and chronic exposure to cyanide (Philbrick et al. 1979). In humans, however, only a few cases of permanent neurological lesions have been reported after cyanide exposure as a result of suicide attempts (Uitti et al. 1985), treatment with sodium nitroprusside (Kim et al. 1982), laetrile consumption (Kalyanaraman et al. 1983), and occupational exposure (Sandberg 1967). The normal outcome of a non-lethal exposure to cyanide is in fact complete recovery. It is thus unlikely that neurological lesions, other than anoxic damage (Brierley et al. 1976), can be an effect of cyanide alone.

The neurological lesions attribted to cyanide exposure have in fact been attributed to the combined effects of cyanide exposure and metabolic abnormalities affecting the cyanide detoxification mechanisms. Wilson has (1963; 1965) suggested that Leber's hereditary optic atrophy is due to cyanide exposure in the presence of an inborn error of cyanide metabolism. This proposal has recently been supported by the finding of reduced activity of rhodanese in cases of this disease (Cagianut et al. 1981). Hydroxycobalamin deficiency has also been proposed as a predisposing factor for neurotoxic effects of cyanide exposure (Wilson et al. 1971), but prolonged cyanide exposure did not produce neurological changes in baboons on low cobalamin diet (Crampton et al. 1979).

A deficient intake of sulphur aminoacids was likely in the presently studied population since they ate almost only cassava during the drought and cassava contains few sulphur aminoacids (Thampan 1979). In the follow-up studies the intake of sulphur aminoacids was assessed as the urinary excretion of total sulphur and inorganic sulphate as proposed by Sabry et al. (1965). Serum and urinary sulphur aminoacid levels have been used to measure the intake of these compounds in cassava-eaters (Osuntokun et al. 1968b; Vis et al. 1982), but determination of sulphur metabolites was considered preferable due to

its simplicity, low cost, and the great stability of sulphate (Lundquist et al. 1980). The excretion of inorganic sulphate in children from the affected area was less than 20% of that found in Swedish controls. It was also lower than that reported from rural children in Kenya with diets deficient in sulphur aminoacids (Simmons 1973).

During the drought it was observed that the non-affected population in the coastal zone ate more supplementary foods, mainly fish, than did the affected populations living further inland. In the follow-up studies children from the affected area also had a lower excretion of total sulphur and inorganic sulphate compared to children from the coastal zone (Table IV). The very low excretion of inorganic sulphate indicates that the diet of the affected population was characterized by a very low sulphur intake. The population in the coastal zone also consumed cassava but they had both a lower cyanide intake and a higher sulphur intake. The results are thus consistent with the hypothesis that the neurological lesions were caused by an association of high cyanide and low sulphur intake.

It has been suggested (Osuntokun et al. 1968b) that neurological lesions attributed to cassava consumption are a direct effect of cyanide and that sulphur deficiency may be a contributory factor by reducing the cyanide detoxification capacity. Another possible mechanism is that the preferential use of the limited amounts of sulphur aminoacids for cyanide detoxification leads to impaired synthesis of sulphur compounds essential to the nervous system as suggested in paper III. The first hypothesis would imply that blood cyanide levels are increased due to reduced detoxification resulting from deficiency of sulphur. The hypothesis that the neurological lesions are caused by deficiency of essential sulphur compounds does not require increased blood cyanide levels. Blood cyanide levels are thus of uttermost interest to study but the instability of cyanide in blood did not allow

the transort of specimens and no determination of blood cyanide could be done in the study population.

To facilitate the determination of blood cyanide in remote rural areas, it was necessary to develop a new method which enabled the transport of specimens for later determination of blood cyanide. The study presented in paper IV demonstrates that the addition of silver ions stabilized whole blood cyanide at all temperatures studied. As most of the cyanide in blood is trapped in the erythrocytes, determination of whole blood cyanide in specimens treated with silver ions is suggested for studies on cyanide levels in field surveys. Silver ions also had a stabilizing effect on cyanide in plasma. In the absence of these ions, cyanide reacts rapidly with a compound in the albumin fraction of blood, possibly with cystine residues in albumin yielding iminothiazolidine carboxylic acid (or amino-thizolinecarboxylic acid) (Wood & Cooley 1956). Of interest is that this compound has been demonstarated in the urine of animals receiving high doses of cyanide. It may thus be an appropriate indicator of high blood cyanide levels over a period of days, if better determination methods can be developed.

Although a combination of high cyanide and low sulphur intake is the most likely mechanism behind the spastic paraparesis observed, other etiologies must be considered. Thus Lathyrism is a disease that occurs as epidemics of spastic paraparesis (Spencer 1983) in drought affected areas due to high consumption of the drought resistant pea, lathyrus sativa. Its predilection for adolescent men and absence of dysarthria as well as of visual disturbances distinguishes it from the disease in the present study. In fact, the pea that causes lathyrism was not consumed in the affected part of Mozambique. Wild plants, however, were eaten in large amounts by the affected population during the drought (Casadei et al. 1984) but none of the plants identified are known to produce the type of neurological lesions observed. Neurotoxic effects on

the spinal cord, occurring as epidemics, have been reported after exposure to insecticides (Vasilescu et al. 1984) and as an adverse effect of treatment with halogenated hydroxyquinolines (Baumgartner et al. 1979), but no such exposure could be verified in the population studied.

Infectious etiology is improbable in the disease under study because no general symptoms supported this etiology. There was no increase of cells in CSF, and tests for virus were negative. Of some interest in this context is the suggestion of viral etiology in neurological disorders, including spastic paraparesis, observed in cases with acquired immunodeficiency syndrome (AIDS) (Shaw et al. 1985; Levy et al. 1985). Selective nutritional deficiency has, however, also been suggested as the etiology of these neurological disorders, all of which occured in cases with severe signs of AIDS, including weight loss (Petito et al. 1985). No signs of AIDS were observed in the cases in the present study, nor in the cases examined one or two years later, and the observed disease must be regarded as unrelated to AIDS. Of certain interest is also the fact that antibodies reacting with human T-lymphotropic virus type-I (HTLV-I) has recently been reported in about 60% of cases with tropical spastic paraparesis in Martinique (Gessain et al. 1985), Jamaica and Colombia (Rodgers-Johnson et al. 1985). The significance of these observations is yet unclear, and it should also be noted that the paraparesis described from Central America differed from the presently studied disease by its insidious onset, in an older age-group.

High exposure to cyanide may have other consequences and an abnormal thyroid function could be expected in the population affected by the epidemic due to the interference with iodine transport in the thyroid gland by the high levels of thiocyanate observed (Green 1978). Serum levels of TSH were raised, but no hypothyroid values were found. The T3/T4 ratio was increased, and in combination with increased TSH, this is a typical pattern found in populations suffering from iodine

deficiency (Chopra 1975). However, the studied population had an adequate iodine intake as indicated by normal urinary levels of iodine. The goitre rate was low and corresponded to the sporadic goitre found in populations on adequate iodine intake (Thilly et al. 1980). The results thus indicate an adaptation of the thyroid function to the heavy thiocyanate exposure, and that the adequate iodine intake, in addition to this adaptation, has protected the population against the development of overt hypothyroidism and goitre. This is in accordance with earlier studies on cassava-eaters indicating that the thyroid function depends upon the dietary balance between iodine and thiocyanate (Delange 1983).

Measures to prevent presumed toxic effects must not counteract the positive health effects of cassava (Okigbo 1980). One of the most important advantages of cassava cultivation became apparent during the present study, namely that the studied population survived the drought due to a reasonable harvest of toxic cassava, when all other crops had failed. The population was aware of the toxicity of cassava. Nevertheless they ate the toxic roots as they had no other possibility to survive. The extensive cultivation of toxic cassava varieties in the area proved to be a safeguard against famine, saving thousands from dying of starvation. In such a situation the promotion of efficient and rapid methods to reduce the cyanide content of cassava roots is obviously the most appropriate measure to reduce cyanide exposure. It should be noted that considerable cyanide exposure persisted in the cassava harvest periods following the drought, as did a low intake of sulphur amino-acids. In spite of this, only sporadic cases of paraparesis were observed during these periods. It thus appears that a possible "trigger point" at which high cyanide and low sulphur intake may elicit severe neurological damage is reached only under the most severe circumstances. The thyroid gland was also found to adapt to considerable exposure to thiocyanate, in the presence of an adequate iodine intake, without the development of goitre and cretinism.

As cassava constitutes a staple crop with high yields and good resistance to drought and pests, a reduction of cassava cultivation due to fear of toxic effects may in fact impair the health of millions of poor peasant families growing this crop. On the other hand, by raising agricultural productivity, an increased cultivation of cassava can improve health (Omawale & Rodrigues 1980), provided that efficient processing methods are used to reduce its cyanide content, that protein-rich supplementary foods are available, and that iodine supplementation is given when necessary. Trade should be developed in order to exchange a surplus of cassava or other cash crops for protein-rich supplements. Prolonged breastfeeding (Omawale & Rodrigues 1980) and adequate weaning foods (Cameron & Hofvander 1980) should be promoted in these areas.

Finally it should be remembered that caloric intake is still a limiting factor in the diet of many people in the least developed countries (LDCs). The agricultural production growth rate in most LDC's in sub-Saharan Africa is about 2%, whereas the rate of population growth is 3-4% (Okezie 1982). In many of these countries cassava can play a very important role in increasing agricultural production. Only under very special circumstances are toxic effects to be feared. Still it is important to elucidate these effects in order to make full advantage of the agricultural potential that cassava offers to the poor peasants. Safe cyanide levels in cassava products should be defined (Seneviratne 1985) and appropriate analytical methods developed to monitor these levels. Populations on cassava-dominated diets should be surveyed for neurological disorders and goitre(Delange et al. 1983). Chemical screening methods should be developed and used to estimate cyanide exposure and its effect in these populations. The present study suggests that examination of the ankle clonus rate, determination of urinary excretion of inorganic sulphate and thiocyanate, as well as determination of cyanide in blood after stabilization with silver ions could be useful in this context.

GENERAL SUMMARY

1. An epidemic of spastic paraparesis occurred in June-October 1981 in a drought-stricken rural area of Mozambique.

2. Cassava was the only crop to survive the drought, which induced abnormally high cyanide levels in the root.

3. Due to shortage of other foods, the roots were eatan without the sun-drying normally used to reduce cyanide levels.

4. High cyanide intake was verified by high cyanide levels in food and increased thiocyanate levels in serum of cases.

5. Low intake of sulphur, needed for cyanide detoxification, was verified by low urinary excretion of inorganic sulphate.

6. It is suggested that the neurological damage was caused by the combination of high cyanide and low sulphur intake.

7. A method for determination of blood cyanide based on the stabilizing effect of silver ions was developed for further field studies.

8. Ankle clonus in apparently healthy children was related to cyanide exposure from cassava and may indicate minor lesions to cortico-spinal tracts.

9. In the presence of an adequate iodine intake, goitre and hypothyroidism did not develop in spite of high thiocyanate loads from cassava.

10. Cassava saved the study population from starvation during the drought and measures to avoide presumed toxicity must not counteract the positive effects of the crop.

ACKNOWLEDGEMENTS

This study consists of investigations done in collaboration with colleagues from the Ministry of Health in Mozambique, and laboratory investigations mainly done at the Department of Clinical Chemistry, Linköping University, Sweden. Without the collaboration and assistance of many people, the study would never have been accomplished. This especially applies to the Mozambican peasants, who participated in the study, in a very difficult situation.

My special thanks are due to:

The Minster of Health of Mozambique, Dr Pascoal Mocumbi, as well as Dr Jorge Cabral and Dr Ana Maria Nóvoa at the National Directorate of Preventive Medicine for great interest and personal guidance of this study.
Julie Cliff, coordinator of the studies on the epidemic, for invaluable and stimulating cooperation.
Anne Rodrigues, Enri Casadei, Nicolai Kulinsky, and Sander Essers for fruitful cooperation.
Lucas Chomera, Provincial Director of Health, and Hilario Candido, Lucia Bogliotti, Alberto Venancio Jeque, Antonio Esteves, Nafissa Osman, Ilidio Manjate, Elena Madi, all health workers in Nacala-Porto, as will as many other health workers and community leaders in the Province of Nampula for the most stimulating teamwork I have ever experienced.
Anders Molin for all collaboration and unfailing friendship.

Yngve Hofvander, for constant support, encouragement, and constructive criticism.
Bo Sörbo for his invaluable contributions, enthusiastic teaching and never-failing interest in the present study.
Harry Boström for enthusiasm and excellent guidance.
Pontus Wiklund for encouragement and decisive support.
Per Lundquist for inspiring collaboration and skillful technical assistance.

Leif Wide, Johannes Mårtensson, Rolf Jansson, Mohamed Eltom, and Jerker Liljestrand for valuable criticism.
Åke Bruce and Ia Torelm for advice and assistance.
The entire staff and all co-workers of the International Child Health Unit for support and a most stimulating atmosphere.
Christel Richter, Gunvor Söderström, Inga Hellman, and Laila Grandin for secretarial and administrative help.
Donald MacQueen for revising the language and Martin Lidholt for making the drawings.
Karin Wennqvist, as well as the staff of the Medical Library for all assistance.
Tore Lundström for advice on statistics and computerizing.
Inger Sörbo and other friends in Linköping as well as in Mozambique for generous hospitality.
My children, parents, and parents-in-law for all support.
Finally my wife Agneta who throughout these studies has been my frankest critic and main support - in fieldwork as well as in housework.

These studies were supported by grants from the Maude and Birger Gustavsson Foundation, by grant No. 83/204, from the Swedish Agency for Research Cooperation with Developing Countries, and by grant No. 03X-05644 from the Swedish Medical Research Council.

REFERENCES

Amaral E, Casadei E, Cliff J, Figueiredo J. Evoloção de teor em tiocianáto em urinas de uma população exposta a intoxicação cronica por ácido cianidrico. In: Journadas de Saude V, Ministério de Saúde, Moçambique, 1985.

Anonymous. Surveillance of peripheral neuropathies. Wkly Epidem rec 1982; 57: 213-4.

Barrett MD, Hill DC, Alexander JC, Zitnak A. Fate of orally dosed linamarin in the rat. Can J Physiol Pharmacol 1977; 55: 134-6.

Baumgartner G, Gawell MJ, Kaeser HE et al. Neurotoxicity of halogenated hydroxyquinolines: clinical analysis of cases reported outside Japan. J Neurol Neurosurg Psychiatry 1979; 42: 1073-83.

Bourdoux P, Delange F, Gerard M, Mafuta M, Hanson A, Ermans AM. Evidence that cassava ingestion increases thiocyanate formation: a possible etiological factor in endemic goiter. J Clin Endocrinol Metab 1978; 46: 613-21.

Bourdoux P, Seghers P, Mafuta M et al. HCN content and detoxification processes. In: Delange F, Iteke FB, Ermans AM eds. Nutritional factors involved in the goitrogenic action of cassava. Ottawa, Canada: Int Develop Res Centre Monogr 1982; IDRC-184e: 51-8.

Boxer GE, Richards JC. Determination of traces of hydrogen cyanide in respiratory air. Arch Biochem Biophys 1952; 39: 287-91.

Boyland E, Walker SA. Effect of thiocyanate on nitrosation of amines. Nature 1974; 248: 601-2.

Braganca A, First R, eds. A transformação da Agricultura familiar na Província de Nampula. Maputo: Universidade Eduardo Mondlane, Centro de estudos Africanos, CEA Relatorio No. 80/3, 1980.

Brierley JB, Brown AW, Calverley J. Cyanide intoxication in the rat: physiological and neuropathological aspects. J Neurol Neurosurg Psychiatry 1976; 39: 129-40.

Brimer L, Christensen SB, Mölgaard P, Nartey F. Determination of cyanogenic compounds by thin-layer chromatography. J Agric Food Chem 1983; 31: 789-93.

Brunnemann KD, Louis Y, Hoffman D. Chemical studies on tobacco smoke. XLIX. Gas chromatographic determination of hydrogen cyanide and cyanogen in tobacco smoke. J Analyt Toxicol 1977; 1: 38-42.

Cagianut B, Rhyner K, Furrer W, Schnebli HP. Thiosulphate-sulphur transferase (rhodanese) deficiency in Leber's hereditary optic atrophy. Lancet 1981; 2: 981-2.

Cameron M, Hofvander Y. Manual on feeding infants and young children. Oxford: Oxford University Press, 1983.

Carvalho M. Agricultura traditional de Moçambique. Lourenço Marqués: Missão de inquerito agricola de Moçambique, 1969; 1-67.

Casadei E, Cliff J, Jansen P et al. "Mantakassa" Uma epidemia de neuropatia tropical associada com intoxicação crónica por cianeto, em área de consumo de mandioca na Província de Nampula, Moçambique. Rev Med Mocambique 1984; 2: 1-34.

Cattaneo A. Relatorio sobre os casos de edema em adultos surgidos no Distrito de Memba. Maputo: Ministerio de Saude, 1982.

Chen KK, Rose CLL. Nitrite and thiosulphate therapy in cyanide poisoning. JAMA 1952; 149: 113-9.

Cheok SS. Acute cassava poisoning in children in sarawak. Trop Doct 1978; 8: 99-101.

Chopra IJ, Hershman JM, Hornabrook RW. Serum thyroid hormone and thyrotropin levels in subjects from endemic goiter regions of New Guinea. J Clin Endocrinol Metab 1975; 40: 326-33.

Clark A. Aetiology of pellagra and allied nutritional diseases. West Afr Med J 1935; 8: 7-9.

Cock JH. Cassava: A basic energy source in the tropics. Science 1982; 218: 755-62.

Cooke RD, Coursey DG. Cassava: A major cyanide containing food crop. In: Vennesland B et al. Cyanide in biology. New York: Academic Press, 1981.

Cosnett JE. Unexplained spastic myelopathy. S Afr Med J 1965; 39: 592-5.

Coursey DG. Cassava as food: Toxicity and technology. In: Nestel B, MacIntyre R eds. Chronic cassava toxicity. Ottawa, Canada: Int Develop Res Centre Monogr 1973; IDRC-010e: 27-36.

Crampton RF, Gaunt IF, Harris R et al. Effects of low cobalamin diet and chronic cyanide toxicity in baboons. Toxicology 1979; 12: 221-34.

Cruickshank EK. Effects of malnutriton on the central nervous system and the nerves. In: Handbook of clinical neurology. Vol. 28. Metabolic and deficiency diseases of the nervous system, Part 2. Amsterdam: Elsevie North Holland 1976: 1-41.

Cutting WAM. Cassava - a valuable food but a possible poison. Trop Doct 1978; 8: 102-3.

De Bruijn GH. The cyanogenic character of cassava (Manihot esculenta). In: Nestel B, MacIntyre R eds. Chronic cassava toxicity. Ottawa, Canada: Int Develop Res Centre Monogr 1973; IDRC-010e: 43-8.

Delange F, Bourdoux P, Colinet E et al. Nutritional factors involved in the goitrogenic action of cassava. In: Delange F, Ahluwalia R eds. Cassava toxicity and the thyroid: Research and public health issues. Ottawa, Canada: Int Develop Res Centre Monogr; 1983; IDRC-207e: 17-26.

Denny-Brown D. Neurological conditions resulting from prolonged and severe dietary restriction. Medicine 1947; 16: 41-113.

Dwivedi MP, Prasad BG. An epidemiological study of lathyrism in the district of Rewa, Madhya Pradesh. Ind J Med Res 1964; 52: 81-116.

Ebrahim GJ, Haddock DRW. Polyneuropathy of probable nutritional origin in Dar-es-Salaam, Tanganyika. Trans R Soc Trop Med Hyg 1964; 58: 246-54.

Ekpechi OL, Dimitriadou A, Fraser R. Goitrogenic activity of cassava(a staple Nigerian food). Nature 1966; 210: 1137-8.

Eltom M, Salih MAM, Boström H, Dahlberg PA. Differences in aetiology and thyroid function in endemic goitre between rural and urban areas of the Darfur region of the Sudan. Acta Endocrinol 1985; 108: 356-60.

Ermans A M, Kinthaert J, Van der Velden M, Bourdoux P. Studies of the antithyroid effects of cassava and thiocyanate in rats. In: Ermans AM et al. eds. Role of cassava in the etiology of endemic goitre and cretinism. Ottawa, Canada: Int Develop Res Centre Monogr 1980; IDRC-136e: 93-110.

FAO/UNDP/MOC. Esboco dos recursos agrarios de Mocambique. Carta 3: Esboco das zonas agro-climaticas. Maputo, 1980.

Frankenberg L, Sörbo B. Effect of cyanide antidotes on the metabolic conversion of cyanide to thiocyanate. Arch Toxicol 1975; 33: 81-9.

Gebre-ab T, Gabriel ZW, Maffi M, Ahmed Z, Ayele T, Fanta H. Neurolathyrism - A review and a report of an epidemic. Ethiop Med J 1978; 16: 1-11.

Gill GV, Bell DR. Persisting nutritional neuropathy amongst former war prisoners. J Neurol Neurosurg Psychiatry 1982; 45: 861-5.

Gessain A et al. Antibodies to human t-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet 1985; ii: 407-10.

Green WL. Mechanism of action of antithyroid compounds. In: Werner SC and Ingbar SH, eds. The Thyroid. 4th ed New York: Hasper and Row Publishers, 1978; 77-87.

Hagbarth KE. Muscle spindle discharge patterns in tremor and clonus. In: Findley LJ, Capildeo F, eds. Movement disorders: Tremor. London: The Macmillan Press, 1984: 157-64.

Horowitz W ed. Official methods of analysis of the association of official chemists. Washington: Association of official chemists, 1980; 433.

Jones WO. Manioc in Africa. Stanford, Calif.: Stanford University Press; 1959.

Kalyanaraman UP, Kalyanaraman K, Cullinan SA, McLean JM. Neuromyopahy of cyanide intoxication due to "Laetrile" (Amygdalin). Cancer 1983; 51: 2126-33.

Kim YH, Foo M, Terry RD. Cyanide encephalopathy following therapy with sodium nitroprusside. Arch Pathol Lab Med 1982; 106: 392-3.

Kingsley M. Travels in West Africa. London: Virago Press, 1982.

Labianca DA. On the nature of cyanide poisoning. J Chem Educ 1979; 56: 788-91.

Lancaster PA, Brooks JE. Cassava leaves as human food. Econ Botany 1983; 37: 331-48.

Lessell S. Experimental cyanide optic neuropathy. Arch Ophtalmo 1971; 86: 194-204.

Levy JA, Shimabukuro J, Hollander H, Mills J, Kaminsky L. Isolation of AIDS-associated retroviruses from cerebrospinal fluid and brain of patients with neurological symptoms. Lancet 1985; ii: 586-8.

Lucasse C. Le "Kitondji". Une paralysie spastique. Ann Soc Belge Med Trop 1952; 32: 391-401.

Lundquist P, Mårtensson J, Sörbo B & Öhman S. Method for determining thiocyanate in serum and urine. Clin Chem 1979; 25: 678-81.

Lundquist P, Mårtensson J, Sörbo B, Öhman S. Turbidimetry of inorganic sulfate, ester sulfate and total sulfur in urine. Clin Chem 1980; 26: 1178-81.

Lundquist P, Mårtensson J, Sörbo B, Öhman S. The adsorption of thiocyanate by anion-exchange resins and its analytical application. Clin Chem 1983; 29: 403.

Makene WJ, Wilson J. Biochemical studies in Tanzanian patients with ataxic tropical neuropathy. J Neurol Neurosurg Psychiatry 1972; 35: 31-3.

Mani KS, Mani AJ, Montgomery RD. A spastic paraplegic syndrome in South India. J Neurol Sci 1969; 9: 179-99.

McDowell J. In defence of African foods and food practices. Trop Doct 1976; 6: 37-42.

McMillan DE, Geevarghese PJ. Dietary cyanide and tropical malnutrition diabetes. In: Podolsky S, Viswanathan M. Secondary Diabetes: The spectrum of the diabetic syndromes. New York: Raven Press, 1980: 239-47.

Ministério da Saúde. Cuidados de saúde primários em Moçambique. Ministério de Saúde, Maputo, 1980.

Moertel CG, Ames MM, Kovach JS, Moyer TP, Rubin JR, Tinker JH. A pharmacologic and toxicological study of amygdalin. JAMA 1981; 13: 591-4.

Moertel CG, Fleming TR, Rubin J et al. A clinical trial of amygdalin (leatrile) in the treatment of human cancer. New Engl J Med 1982; 306: 201-6.

Monekosso GL, Wilson J. Plasma thiocyanate and vitamin B12 in Nigerian patients with degenerative neurological disease. Lancet 1966; i: 1062-4.

Montgomery RD, Cruickshank EK, Robertson WB, McMenemey WH. Clinical and pathological observations on Jamaican neuropathy. Brain 1964; 87: 425-69.

Nartey F. Cyanogenesis in tropical feeds and foodstuffs. In: Vennesland B et al. Cyanide in biology. New York: Academic Press, 1981.

Nkamany K, Kayinge K. Report of mission on spastic paralysis in the valley of rivers Lukula, Luie and Inzia in Bandundu. Dept of Public Health, National Nutrition Planning Center (CEPLANUT), Zaire. Report No 30, 1982.

Odell WB, Wilber JF, Paul WE. Radioimmunoassay of thyrotropin in human serum. J Clin Endocrinol Metab 1965; 25: 1179-88.

Oke OL. Some aspects of the role of cyanogenic glycosides in nutrition. World Rev Nutr Diet 1979; 33: 70-103.

Okezie BO, Kosikowski FV. Cassava as a food. CRC Critical Reviews in Food Sience and Nutrition 1982; 17: 259-75.

Okigbo BN. Giving high priority to the production of staples of low nutritive quality: The case for cassava (Manihot esculenta, Crantz) in the humid tropics of West Africa. Food Nutr Bull 1980; 2: 1-10.

Omawale, Rodrigues AM. Nutrition considerations in a cassava production program for Guyana. Ecol Food Nutr 1980; 10: 87-95.

Osuntokun BO. An ataxic neuropathy in Nigeria: A clinical, biochemical and electrophysiological study. Brain 1968a; 91: 215-48.

Osuntokun BO Durowoju JE, McFarlane H, Wilson J. Plasma amino-acids in the Nigerian nutritional ataxic neuropathy. Br Med J 1968b; 3: 647-9.

Osuntokun BO, Monekosso GL, Wilson J. Relationship of a degenerative tropical neuropathy to diet, report of a field survey. Br Med J 1969; 1: 547-50.

Osuntokun BO, Aladetoyinbo A, Adeuja AOG. Free-cyanide levels in tropical ataxic neuropathy. Lancet 1970; ii: 372-3.

Osuntokun BO. Cassava diet, chronic cyanide intoxication and neuropathy in the Nigerian Africans. World Rev Nut Diet 1981; 36: 141-73.

Peréz C, Scrimshaw NS, Munoz JA. Technique of endemic goitre surveys. In: Endemic Goitre. WHO Monogr Ser 1960; 44: 369-83.

Petito CK, Navia BA, Cho ES, Jordan BD, George DC, Price RW. Vacuolar myelopathy pathologically resembling subacute combined degeneration in patients with the acquired immunodeficiency syndrome. N Engl J Med 1985; 312: 874-9.

Pettigrew AR, Fell GS. Simplified colorimetric determination of thiocyanate in biological fluids, and its application to investigation of the toxic amblyopias. Clin Chem 1972; 18: 996-1000.

Philbrick DJ, Hill DC, Alexander JC. Physiological and biochemical changes associated with Linamarin administration to rats. Toxicol Applied Pharmacol 1977; 42: 539-51.

Philbrick DJ, Hopkins JB, Hill DC, Alexander JC, Thomson RG. Effects of prolonged cyanide and thiocyanate feeding in rats. J Toxicol Environ Health 1979; 5: 579-92.

Rodgers-Johnson P, Gajdusek DC, Morgan OSC, Zaninovic V, Sarin PS, Graham DS. HTLV-I and HTLV-III antibodies and tropical spastic paraparesis. Lancet 1985; ii: 1247-8.

Roman GC, Roman LN, Spencer PS, Schoenberg BS. Tropical spastic paraparesis: A neuroepidemiological study in Colombia. Ann Neurol 1985; 17: 361-5.

Rubino MJ, Davidoff F. Cyanide poisoning from apricot seeds. JAMA 1979; 241: 359.

Sabry ZI, Shadarevian SB, Cowan JW, Campbell JA. Relationship of dietary intake of sulphur amino-acids to urinary excretion of inorganic sulphate in man. Nature 1965; 206: 931-3.

Sacks BI. Recovery of iodide fro anion exchange resins. Nature 1964; 202: 899.

Sandberg CG. A case of chronic poisoning with potassium cyanide? Acta Med Scand 1967; 181: 233-6.

Sandell EB, Kolthoff IM. Micro determination of iodine by a catalytic method. Microchem Acta 1937; 1: 9-25.

Schulz V, Bonn R, Kindler J. Kinetics of elimination of thiocyanate in 7 healthy subjects and in 8 subjects with renal failure. Klin Wochenschr 1979; 57: 243-7.

Schulz V, Löffler A, Gheorghiu T. Resorption von Blausäure aus Leinsamen. Leber Magen Darm 1983; 13: 10-4.

Schulz V. Clinical pharmacokinetics of nitroprusside, cyanide, thiosulphate and thiocyanate. Clin Pharmacokinetics 1984; 9: 239-51.

Seneviratne G. Making cassava a safer food. Development Forum 1985; 13: 13.

Shaw GM, Harper ME, Hahn BH et al. HTLV-III infection in brains of children and adults with AIDS encephalopathy. Science 1985; 227: 177-82.

Shragg TA, Albertson TE, Fisher CJ. Cyanide poisoning after bitter almond ingestion. West J Med 1982; 136: 65-9.

Simmons WK. Use of the inorganic sulfate sulfur/creatinine ratio in field studies. Am J Clin Nutr 1973; 26: 72-6.

Singh JD. The teratogenic effects of dietary cassava on the pregnant albino rat: a preliminary report. Teratology 1981; 24: 289-91.

Spencer PS, Schaumburg HH. Lathyrism: A neurotxic disease. Neurobehav Toxicol and Teratology 1983; 5: 625-9.

Spillane JD. Tropical neurology. London: Oxford university Press, 1973.

Strachan, H. On a form of multiple neuritis prevalent in the West Indies. Practitioner 1897; 59: 477-84.

Symington IS, Anderson RA, Oliver JS, Thomson I, Harland WA, Kerr W. Cyanide exposure in fires. Lancet 1978; ii: 91-2.

Sörbo B. Thiosulfate sulfurtransferase and mercaptopyruvate sulfurtransferase. In: Greenberg DM, ed. Metabolism of sulfur compounds, Metabolic pathways Vol VII, 3rd ed. New York: Academic press, 1975: 433-56.

Thampan PK. Cassava. Trivandrum, India: Kerala Agricultural University, 1979.

Thilly CH, Delange F, Stanbury JB. Epidemiological surveys in endemic goitre and cretinism. In: Stanbury JB, Hetzel BS eds. Endemic goitre and cretinism; iodine nutrition in health and disease. New York: Wiley medical publications, 1980.

Trolli G. Résumé des observations réunies, au Kwango, au sujet de deux affections d'origine indéterminée 1. Paraplégie spastique épidémique. 2. Syndrome oedémateux, cutané et dyschromique. Brussels: Fonds Reine Elisabeth 1938.

Trolli G. "Konzo," Epidemic spastic paraplegia of the Congo. Trop Dis Bull 1939; 36: 501-2.

Uitti RJ, Rajput AH, Ashenhurst EM, Rozdilsky B. Cyanide-induced parkinsonism: A clinicopathologic report. Neurology 1985; 35: 921-5.

UNICEF. The state o the world's children 1985. Oxford: Oxford University Press, 1984.

Vasilescu C, Alexianu M, Dan A. Delayed neuropathy after organophosphorus insecticide (Dipterex) poisoning: a clinical, electrophysiological and nerve biopsy study. J Neurol Neurosurg Psychiatry 1984; 47: 543-8.

Vesey CJ, Cole PV, Simpson PJ. Cyanide and thiocyanate concentrations following sodium nitroprusside infusion in man. Br J Anaesth 1976; 48: 651-60.

Vesey CJ, Cole PV. Blood cyanide and thiocyanate concentrations produced by long-term therapy with sodium nitroprusside. Br J Anaesth 1985; 57: 148-55.

Wide L. Use of particulate immunosorbents in radioimmunoassay. Methods Enzymol 1981; 73: 203-24.

Villén AF. Contribution à la discussion de la paraplégie spastique épidémique du Kwango. Ann Soc Belg Med Trop 1942; 22: 309-17.

Wilson J. Leber's hereditary optic atrophy: Some clinical and aetiological considerations. Brain 1963; 86: 347-62.

Wilson J. Leber's hereditary optic atrophy: A possible defect of cyanide metabolism. Clin Sci 1965; 29: 505-15.

Wilson J, Linnell JC, Matthews DM. Plasma-cobalamins in neuro-ophtalmological diseases. Lancet 1971; i: 259-61.

COMMUNITY HEALTH CELL
47/1, (First Floor) Sr. Marks Road
Bangalore - 560 001.

Wilson J. Cyanide in human disease: A review of clinical and laboratory evidence. Fundament App Toxicol 1983; 3: 397-9.

Winek CL, Fusia E, Collom WD, Shanor S. Cyanide poisoning as a mode of suicide. Forensic Sci 1978; 11: 51-5.

Vis HL, Vuye A, Hennart P. Serum levels of free amino acids in mothers at delivery, newborns, and adult males. In: Delange F, Iteke FB, Ermans AM eds. Nutritional factors involved i the goitrogenic action of cassava. Ottawa, Canada: Int Develop Res Centre Monogr 1982, IDRC-184e: 70-3.

Wood JL, Cooley SL. Detoxication of cyanide by cysteine. J Biol Chem 1956; 218: 449-57.

Zaninovic V, Biojo R, Barreto P. Paraparesia espástica del Pacífico. Colombia Medica 1981; 12: 111-7.

ACTA UNIVERSITATIS UPSALIENSIS
Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine
Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is either a complete monograph or a joint summary of a number of papers. A few copies of these papers are kept together with their summary at major Swedish research libraries, while the summary alone is more widely spread over the world. The aim of the series *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* is to collect these summaries in order to give them a bibliographical and distributional platform. (Until July 1985 the series was named "Abstracts of Uppsala Dissertations from the Faculty of Medicine").

Distributor:
Almqvist & Wiksell International
Stockholm, Sweden

Tryckkontakt, Uppsala 1986

ISSN 0282-7476
ISBN 91-554-1798-1

Bulletin of the World Health Organization, 62 (3): 477-484 (1984)

© World Health Organization 1984

Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 1. Epidemiology and clinical and laboratory findings in patients*

MINISTRY OF HEALTH, MOZAMBIQUE[1]

Investigation of an epidemic of more than 1000 cases of spastic paraparesis in a drought-striken cassava staple area of Mozambique strongly suggests an association between this disease (called mantakassa), chronic cyanide intoxication, and cassava consumption. In previous reports of neurological disease with similar associations, the disease affected an older age group with usually a gradual onset, and the predominant clinical feature was an ataxic neuropathy. In mantakassa the onset was acute, and mostly women of reproductive age and children were affected. Serum thiocyanate levels in these patients were much higher than previously reported; while spastic paraparesis of unknown etiology occurs in many tropical countries, it has not previously been linked with raised thiocyanate levels. The present evidence linking cassava consumption to the disease is circumstantial, and dietary deficiency is also probably involved. Cassava is an important food crop and a major source of energy for people in many parts of the world. In these areas, when there is a shortage of food production, e.g., during a drought, the inhabitants may be exposed to the risk of dietary cyanide intoxication. Further research is needed on the causes of mantakassa so that a repetition of this disaster could be prevented.

This report describes the investigation of an outbreak of more than 1000 cases of spastic paraparesis of sudden onset which strongly suggests an association between this disease and chronic cyanide intoxication due to cassava consumption. The disease is called by its local name — *mantakassa*, which means paralysis.

First reports of an outbreak of spastic paraparesis came from the remote health centre of Cavá in Nampula Province, northern Mozambique, on 13 August 1981. Over the next two months a team of health workers and scientists from the province and Ministry of Health confirmed the existence of a large epidemic of spastic paraparesis, and investigated its possible causes. The investigation was hampered by the remoteness of the area, lack of transport and other resources, and the need to give priority to relief operations. It was helped by the active collaboration of the political and administrative authorities, local community leaders, and the population.

Although initially it was considered to be probably a viral disease, the people of the area suspected the bitter variety of cassava and further investigations showed that they were probably correct.

The epidemic occurred under conditions of a severe drought; the resulting drop in agricultural production meant that the only food crop to survive the drought in quantity was bitter cassava. In this part of Mozambique, the cyanogenic glycosides in the cassava root are normally removed by drying in the sun and storage, a lengthy process that could not be carried out properly because of the food shortage. Commercial food stocks were scarce and the people had to eat a variety of wild plants as well.

Fig. 1 shows the area affected by the outbreak, with a total population of about 500 000. Most of these people are engaged in cash-crop cultivation and live in scattered dwellings; after the country's independence in 1975, up to 8% of the population have moved to new communal villages.

Although cassava consumption and chronic cyanide intoxication have been reported in association

* Part 2 appears on pages 483-490 in this issue of the *Bulletin*. Requests for reprints should be sent to: Secção de Epidemiologia, Direcção Nacional de Medicina Preventiva, Ministério da Saúde, Maputo, Mozambique.

[1] This text was prepared for the Ministry of Health, Mozambique by *J. Cliff*, Clinical Epidemiologist, National Directorate of Preventive Medicine, Ministry of Health, Maputo, Mozambique; *A. Martelli*, Director, Microbiology Department, Faculty of Medicine, Eduardo Mondlane University, Maputo, Mozambique; *A. Molin* and *H. Rosling*, former District Medical Officers, Nacala Hospital, Nacala, Mozambique.

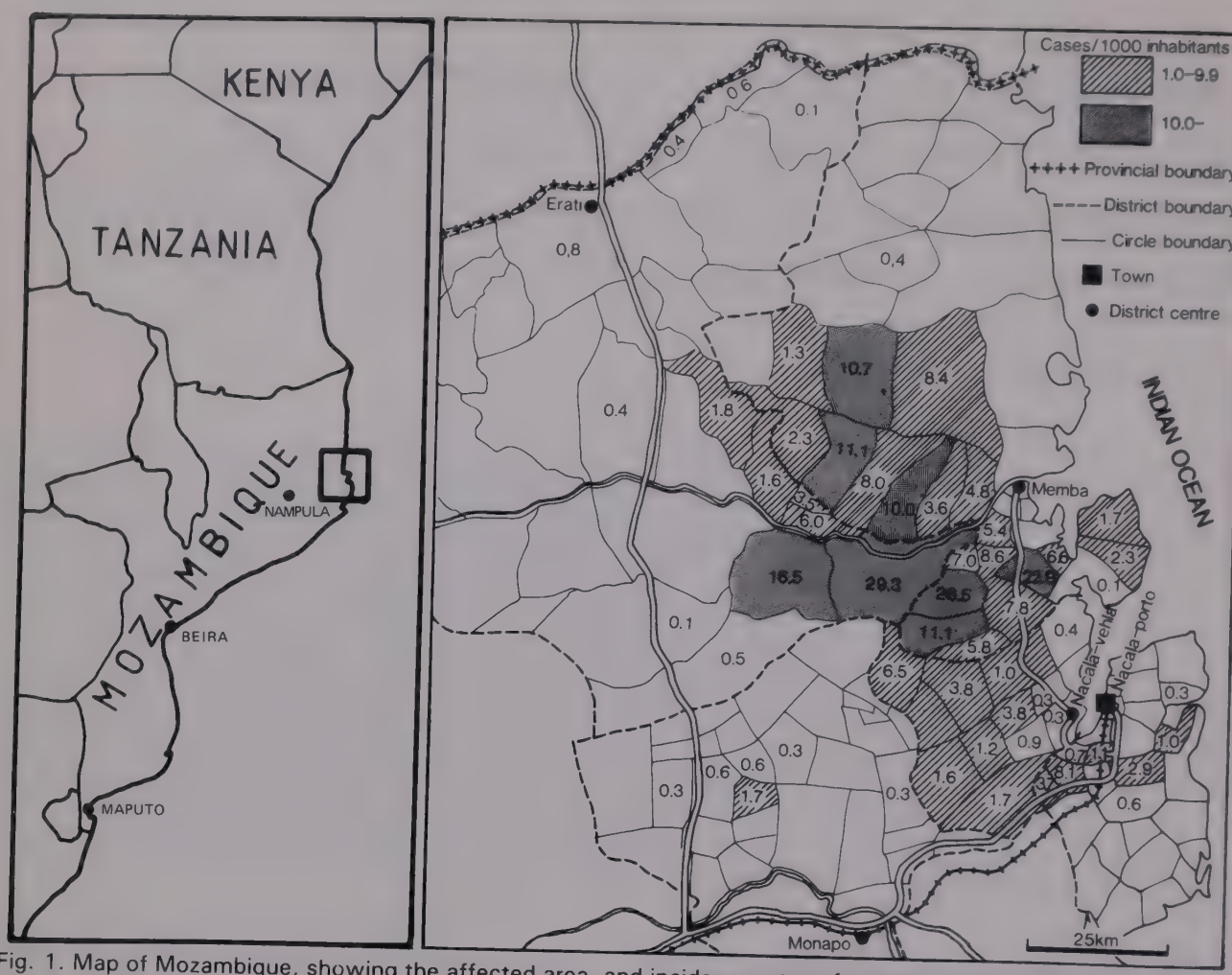


Fig. 1. Map of Mozambique, showing the affected area, and incidence rates of mantakassa per 1000 inhabitants.

with chronic neurological disease (*1*), only one previous report was found of a possible association with an epidemic of acute neurological disease (*2*). Within Mozambique, there is no history of a similar epidemic.

Described below are the results of the epidemiological survey and of clinical and laboratory examinations. A second paper dealing with the agricultural and nutritional situation presents the results of analysis of cassava for cyanide content.

MATERIALS AND METHODS

Epidemiological methods

Because there were few health centres in the affected area, mobile brigades of paramedical workers were formed to search actively for cases. These brigades were reinforced by health workers from other parts of Nampula Province. The brigade members were mostly sanitary agents or nurses, and they travelled by motorcycle or on foot. They covered a wide area, and went well beyond the limits of the area where cases were detected. They began active case detection in late August 1981 and continued until the end of October, by which time there were few new cases. The epidemic is considered to have ended then, although from November onwards small numbers of both new and old cases continued to be reported through a routine notification system that was set up in the health centres.

Active case detection was based on close collaboration with community leaders. Initially the brigades met these leaders, and asked them to look out for patients who had difficulty in walking since the last rainy season (March–April 1981) and to register their names. A brigade member then visited the patients in their homes and recorded on a form such details as age, sex, dwelling place, and symptoms and their time

of onset. The criterion for a case to be included was visible difficulty in walking, as well as increased knee and ankle reflexes. Cases of difficulty in walking due to other diseases such as severe anaemia were excluded. The brigades used the month of Ramadan, which in 1981 was in July, as the main reference point. The predominantly Muslim population of the area remembered it well as the most important religious month of the year.

Most cases were easy to identify, but where doubts existed, a doctor visited the patient. The doctors performed a more thorough neurological examination in doubtful cases and looked for signs such as a sensory level that would suggest a localized cord lesion, and Argyll Robertson pupils that would suggest neurosyphilis. In most cases the diagnosis was confirmed, but in a few cases other neurological diseases common in underdeveloped countries such as poliomyelitis, hydrocephalus and Pott's disease were found. Besides checking on doubtful cases, the doctors routinely examined many other patients as well.

The incidence rate of mantakassa for each political division, based on the number of cases found by active case detection and the estimated population of the division, was calculated. The communal village of Acordo de Lusaka in Memba District was selected for a more detailed investigation, including a case-control study, because it was in an area of high disease incidence and the patients were readily accessible. In every house in the village a population census and case detection were carried out.

Clinical material and methods

During several visits to the affected area between 25 August and 9 October 1981, a neurologist or a physician took the histories of and thoroughly examined a series of 53 patients to obtain as complete a clinical description as possible. Twenty-eight of these patients lived in Acordo de Lusaka which was chosen for detailed investigation; the remainder were either in-patients in the health centres or went there for examination. Thus they were not a representative sample. However the pattern of symptoms present in both groups was similar. Many other patients were also examined but less thoroughly, including six severely blind patients in the health centres. Where appropriate, the findings in these six patients have been included.

The patients examined had been ill for varying lengths of time — from 1 day to 4 months. Some were seen on more than one occasion. History-taking was difficult, both because of the length of time that had often elapsed since the beginning of the illness, and because of difficulties in communication. Translation was necessary from the patients' language to Portuguese, and some symptom concepts did not appear to have an exact translation. Dysarthria was difficult to evaluate because of unfamiliarity with the local language, while testing for visual acuity was based on the ability to see two fingers at a distance.

The health centres were provided with rehabilitation facilities, so that patients treated at these centres received a diet rich in protein and energy, as well as vitamin supplements, a course of hydroxycobalamin injections, and physiotherapy. The effect of this treatment could not be satisfactorily evaluated.

The same physician re-examined 17 of the original 28 patients in the study village, 4 patients in one of the health centres, and 3 of the severely affected blind patients in June 1982.

Laboratory material and methods

Examination of 6 blood films from patients, and 16 samples of cerebrospinal fluid (CSF) for cells and for protein (using Pandy's test) was carried out within Mozambique. Specimens were also collected for later analysis outside Mozambique. Blood samples were collected from patients at ten different sites in the affected area and on three separate occasions — on 27–28 August, 10–12 September, and 5–8 October. In 22 patients the blood was collected twice, and in one patient three times. In the study village, blood was collected from 22 healthy neighbour and family controls, who were age- and sex-matched to the patients as closely as possible.

The first batch of 5 frozen serum specimens and the second batch of 43 heparinized blood specimens from patients were sent to Ruchill Hospital, Glasgow, Scotland. The third batch of 198 heparinized blood specimens from patients and 22 from controls was sent to the World Health Organization in Geneva and stored at 4 °C for 6 months before forwarding to the Regional Hospital, Linköping University, Sweden.

Thirteen urine and 16 CSF speciments were also sent to Glasgow. These, together with 29 serum specimens were tested for viruses at the Regional Virus Laboratory. Ten urine specimens were analysed for pesticides — organophosphates, DDT, and lindane — at the Royal Hospital for Sick Children, Glasgow.

Thiocyanate analyses on the 48 blood and serum specimens from patients were performed at the Royal Hospital for Sick Children, using a modification of Pettigrew's method (*3*). In Sweden, thiocyanate in the samples from patients and controls was analysed on 100 μl of supernatant after centrifugation using the method described by Lundquist et al. (*4*). Thiocyanate was separated from interfering compounds using an ion-exchange resin (Amberlyst A21). By the time of analysis for thiocyanate, 7 of the Glasgow specimens and all the Swedish specimens had haemolysed; 14 of the Swedish specimens showed some mould growth.

Fourteen specimens of frozen sera collected from pregnant women in August 1981 in a non-cassava staple area in central Mozambique were analysed in Sweden using the same method.

RESULTS

Epidemiological findings

Altogether 1102 patients were identified during the course of the epidemic. Fig. 1 shows that they were spread over five districts. The greatest number (772) were in Memba District. The most severely affected areas were the hinterland of Memba District and adjoining parts of Erati District. The central coastal region of Memba District was spared, and few cases were reported from other parts of the coast. No cases were reported from the district capitals. No deaths in patients were reported during the course of the epidemic.

The areas with most cases also had the highest incidence rates. The highest recorded incidence rate found by active case detection was 29 per 1000 inhabitants in one political division. In the village study, 28 patients were found among the 815 inhabitants, giving an incidence rate of 34 per 1000 inhabitants.

The distribution in time of the epidemic according to the patients' histories followed a characteristic curve, with the earliest cases in mid-May, a gradual increase in June and July, a peak in August, and then a steep decline, with few new cases reported in October.

Table 1 shows the age and sex distribution of 764 (out of the 772) cases notified in Memba District. Similar distributions were found in the other districts. The table shows that 65% of the notified cases were children under 15 years of age, with males predominating. At over 15 years of age, females predominated, with most cases aged between 20 and 40 years. Most of the affected women were lactating, but there were no cases of the disease among their babies. The age distribution followed a similar pattern in the village study, but there was no male preponderance among the children.

Many families were found with more than one member affected. In the village study, 11 out of the 28 patients had other affected family members living in the same household.

Clinical findings

History. A history of the onset of the illness was obtained in 49 out of the 53 patients in the series. The main symptom, which was present in all patients, was difficulty in walking; in 35 patients this appeared with sudden onset. Typical histories were that a child went to bed well, or with some general symptom such as fever, and woke up unable to walk; or that a woman fell down while travelling on foot to fetch water (this is a journey of about 20 km in the prevailing drought conditions). Health workers living in the area personally observed a sudden onset in 3 patients. In a further 11 patients the difficulty in walking began more slowly, taking up to a week to develop, while 3 others gave a history of gradual onset lasting more than one week.

General symptoms around the time of onset included fever, pain (especially in the legs), paraesthesiae, headache, dizziness, and vomiting. A variety of more specific nervous system symptoms were often present at the time of onset, while in others they developed later. The most thorough neurological histories were taken in 44 patients; 22 complained of (or their mothers noted) weakness in the arms, 19 had difficulty in speaking, and 11 had difficulty in seeing. Two mothers said that their children had difficulty in hearing.

In no patient was there a history of altered consciousness or disturbed behaviour. Sphincter control was normal except in 2 children.

Examination. No consistent abnormality was found on general examination, although signs of malnutrition, anaemia, and splenomegaly were present in

Table 1. Age and sex distribution of patients in Memba District

Age (years)	Males		Females		Both	
	No.	% of total	No.	% of total	No.	% of total
0–14	330	43	165	22	495	65
⩾ 15	70	9	199	26	269	35
Total	400	52	364	48	764	100

some patients. Ulceration of the lower limbs was a rare finding, but no patient was found with signs of yaws.

Abnormal findings in the cranial nerves were limited to diminished visual acuity and dysarthria. Fundoscopy was normal. The speech in patients who complained of difficulty in speaking was slurred and consistent with a spastic dysarthria. The children with apparent deafness could not be adequately examined.

In the upper limbs, symmetrically increased upper limb reflexes were present in 28 out of the 53 patients. Many had a positive Hoffman's reflex, and the more severely affected had a positive pectoral reflex. No muscular wasting, loss of power, or sensory changes were found.

In the lower limbs all patients showed a symmetrical spastic paraparesis. Some of the patients who had been immobile for a prolonged period showed muscular wasting. All except 4 patients showed increased knee and ankle jerks; 31 out of the 53 patients had ankle clonus. In some of the worst-affected patients, clonus of the lower limbs, which spread to involve the trunk, was provoked by minimal stimulation. The plantar reflex was downgoing whenever it could be elicited. Seven out of the 53 patients had sensory changes, consisting of loss of sensation to light touch and pinprick in the extremities. Proprioception was normal.

Rombergism was absent. Twenty-four out of the 53 patients could not walk without support. All showed an abnormal gait which was typical of spastic paraparesis.

One patient in the series, and another who was examined in one of the health centres showed cerebellar signs consisting of nystagmus, intention tremor, and dysdiadochokinesia.

The disease spectrum varied from slight weakness of the legs with increased lower limb reflexes to paralysis of all four limbs with blindness. A simple scale to classify disease severity was devised and used in the village study. In this village, 8 out of 15 children, but no adults, were classified as having severe disease, suggesting that the children were more severely affected than the adults.

Evolution

The evolution of the disease could not be satisfactorily evaluated. Re-examination of 17 patients in June 1982 in the study village (Acordo de Lusaka) showed little improvement in the neurological signs and function in the upper and lower limbs, especially in the severely affected patients. Most improvement was recorded in the symptoms of dysarthria and diminished vision; 5 out of 7 patients with difficulty in speaking and 5 out of 8 with diminished vision said that they had improved.

Of the 7 patients re-examined in the health centre, only one showed a slight improvement in the neurological signs in the lower limbs. However, 4 out of the 7 patients with dysarthria had recovered completely, as had the 3 patients with blindness. Despite the lack of improvement in the neurological signs in the lower limbs, all these patients had marked functional improvement and were able to walk with the aid of crutches. This functional improvement contrasted with the condition in the patients who had remained in the village, in most cases without any treatment.

Laboratory findings

Two out of the 6 blood films from patients showed signs of iron-deficiency anaemia, while 5 showed eosinophilia. Both these findings are common in rural Mozambique. The 16 samples of CSF showed no increase in cells, and Pandy's test was negative.

Tests on 10 urine samples for pesticides were negative. Twenty-nine serum, 13 urine, and 16 CSF specimens tested for viruses were also negative.

The mean thiocyanate level on 246 specimens of blood and serum from patients was 329 ± 8 μmol/l. The mean value of the 48 specimens analysed in Glasgow was 298 ± 17 μmol/l, and in Sweden 336 ± 9 μmol/l. The normal range of values for the Glasgow laboratory is 10–40 μmol/l for non-smokers, and 70–150 μmol/l for smokers, and for the Swedish laboratory 8–77 μmol/l for non-smokers and 7–237 μmol/l for smokers. Comparison of samples collected 3 weeks apart from 22 patients showed no significant difference. There were no significant age and sex differences, and no correlation between thiocyanate level and disease severity. In only 4 patients was it certain that the blood was collected within 3 days of the onset of symptoms; these 4 patients showed a mean thiocyanate level of 326 μmol/l.

The 22 controls in the study village also showed high levels of thiocyanate with a mean of 288 ± 23 μmol/l. The mean level in patients in the village was 324 ± 18 μmol/l.

Because of the different collection methods and differences in transport and storage conditions, these results must be treated with caution. Most specimens were haemolysed, which may have influenced the results since thiocyanate is not evenly distributed between cells and plasma, while prolonged storage time may have resulted in some enzymatic conversion of thiocyanate to cyanide. None of these factors could have caused increased thiocyanate levels, but they do make it difficult to compare results between individuals and groups. Statistical tests were therefore not applied to the difference in level found between cases and controls in the village study.

The serum thiocyanate level in 14 pregnant women from a non-cassava staple area in Mozambique was 15 μmol/l.

DISCUSSION

Possible disease etiology

Patients with the disease showed extremely high thiocyanate levels, consistent with chronic cyanide intoxication. Of the available food crops and wild plants eaten, cassava was the most likely source of cyanide (see Part 2). In adults, tobacco smoking may have contributed to the raised thiocyanate levels.

In this study, it has not been possible to show that chronic cyanide intoxication was the cause of the disease because the controls also had high thiocyanate levels, and there was no correlation between disease severity and thiocyanate level. However, the epidemiological features of the disease suggest that chronic cyanide intoxication due to cassava consumption probably played a role in the etiology. The geographical distribution of the disease closely followed an area that had been affected by drought in the previous two years, which led to an increased consumption of insufficiently detoxified cassava with a high cyanogenic glycoside content. The distribution of the epidemic in time corresponded to the consumption of fresh cassava that had not undergone the normal lengthy drying process which is necessary for the detoxification of cyanide. The predilection of the disease for women and children could be due to an increased proportion of cassava in their diet (see Part 2).

A dietary deficiency was probably also involved. Thus, the disease spared the coastal areas where people had access to fish, and the towns where they had access to commercially available foods. It showed a predilection for women of reproductive age and children, i.e., population groups especially vulnerable to nutritional deficiency (see Part 2).

Substances necessary for cyanide detoxification and/or central nervous system metabolism could have been deficient. Sulfur-containing amino acids, which are present in protein-rich foods, supply the sulfur necessary for the detoxification of cyanide to thiocyanate. Cyanide intoxication itself could contribute to a deficiency of sulfur and cobalamin, which are used up in the detoxification of cyanide.

Other etiologies have also been considered. Infection is improbable because the clinical picture does not fit any known infectious disease, viral studies were negative, and the sparing of adult men is difficult to explain. No pesticides were being applied in the area, and tests for pesticides in the patients' urine were negative. There was little commercially available food that could have been contaminated by a toxic substance. Consumption of non-traditional medicine is rare in this community since access to health care facilities is limited. Intoxication from the consumption of wild plants is a possibility, but no wild plant was found that could have been consumed by all the patients, and none of the wild plants consumed is known to cause these symptoms. The distribution in families may suggest a genetic predisposition, but the families also ate the same food.

Many features of the disease remain unexplained, such as its sudden onset. Possibly the disease is triggered when a threshold level of thiocyanate is reached, or when a critical combination of cyanide intoxication plus nutritional deficiency occurs.

Pathomorphology

The main clinical findings of spastic paraparesis associated with increased upper limb reflexes and spastic dysarthria indicate a lesion of the pyramidal tracts. The signs were most frequent and most severe in the lower limbs, suggesting that the lower parts of the pyramidal tracts were preferentially affected, and that the lesion ascended with increasing severity. There were also diminished visual acuity, peripheral neuropathy, cerebellar signs, and reports of deafness which show that other parts of the nervous system were affected.

Comparison with other spastic parapareses in the tropics

Spastic paraparesis has been described from many tropical countries, but in most cases the etiology is obscure (*5*). The disease syndrome which most closely resembles mantakassa is the epidemic paraplegia described by Trolli (*2*) in a cassava staple area of Zaire. Epidemics of this disease occurred in 1928, 1932 and 1937 (*6*), and again in 1978–81 between the months of May and August. The disease predominantly affected women and children (*7*). An endemic form of the same disease exists.

Lathyrism may also present as an epidemic acute spastic paraparesis, but it distinguished from mantakassa by the absence of dysarthria, visual abnormalities, and sensory signs. It is commonest in adult males. No evidence of consumption of the lathyrus pea in the affected area could be discovered.

An undiagnosed endemic form of the disease could exist in this area of Mozambique, which is extremely isolated and is short of medical care facilities. Doctors working in the area have previously seen occasional cases of spastic paraparesis of unknown etiology. Neurologists working in the south of Mozambique, where cassava is also consumed, have reported cases of spastic paraparesis of unknown etiology (N. Kulinski, personal communication, 1981).

Comparison with tropical ataxic neuropathy

In Nigeria (*1*) and the United Republic of Tanzania (*8*), ataxic neuropathy has been reported in association with high serum thiocyanate levels in cassava

staple areas. Several features distinguish mantakassa from these neuropathies. Ataxic neuropathy is not epidemic, and most of the patients are older; the disease onset is usually slow and the commonest signs are diminished sensory perception, bilateral optic atrophy, ataxic gait, impaired muscular coordination, bilateral perceptive deafness, and muscular weakness and wasting. The tendon reflexes are exaggerated in only about 20% of patients. These findings contrast with the acute onset in mantakassa, the predominant clinical finding of spastic paraparesis, and the comparative rarity of signs of peripheral neuropathy.

The mean serum thiocyanate level in patients with mantakassa of 329 μmol/l is considerably higher than the mean levels of 126 μmol/l found in Nigerian patients (*1*) and of 93 μmol/l found in Tanzanian patients (*8*).

The acuteness of onset, the occurrence in younger age groups, and the higher serum thiocyanate levels found in mantakassa suggest that the rate of intoxication may be important in determining the clinical expression of chronic cyanide intoxication.

Other diseases associated with chronic cyanide intoxication

Chronic cyanide intoxication due to cassava consumption can cause endemic goitre and cretinism when associated with iodine deficiency (*9*). There were no signs of goitre in this population. Other authors have suggested that diabetes (*10*) and carcinoma (*11*) may result from a high dietary cyanide intake. To detect the presence of these diseases would require a more detailed future study.

CONCLUSION

The results of this investigation suggest an association between epidemic spastic paraparesis, chronic cyanide intoxication, and cassava consumption. The second part of this study examines in more detail the evidence linking chronic cyanide intoxication with cassava consumption.

ACKNOWLEDGEMENTS

The help of many people in this collective work is acknowledged. In the affected districts, community leaders, the administration, and health workers gave unstinting help. In Nampula City, special thanks are due to the provincial health administration. In Maputo, Dr Jorge Cabral and Dr Ana Maria Novoa of the National Directorate of Preventive Medicine gave valuable support. From abroad, help was given for the analysis of specimens by Professor Bo Sörbo and Professor Per Lundquist of the Department of Clinical Chemistry at the University of Linköping, Sweden, and by Dr Robert Logan and Mr J. Kneiple of the Department of Biochemistry, Royal Hospital for Sick Children, Glasgow, Scotland, Dr E. Bell of the Regional Virus Laboratory, Glasgow, and Dr D. Reid of the Communicable Diseases (Scotland) Unit. Dr Nigel Leigh, consultant neurologist at Southampton General Hospital, England, and Dr Lesley Sedal, consultant neurologist at Prince Henry's Hospital, Melbourne, Australia, provided critical comments and advice.

RÉSUMÉ

LE MANTAKASSA: ÉPIDÉMIE DE PARAPARÉSIE SPASMODIQUE ASSOCIÉE À UNE INTOXICATION CYANURÉE CHRONIQUE, DANS UNE RÉGION DU MOZAMBIQUE OÙ LE MANIOC EST L'ALIMENT DE BASE. I. ÉPIDÉMIOLOGIQUE, OBSERVATIONS CLINIQUES ET RÉSULTATS DE LABORATOIRE

Une épidémie de paraparésie spasmodique ou *mantakassa* (de son nom local) est survenue entre les mois de mai et d'octobre 1981 dans un secteur de la province de Nampula (dans le nord du Mozambique) touchée par la sécheresse. La totalité des 1102 malades recensés étaient des paysans, et l'affection a surtout frappé les femmes en âge de procréer et les enfants. La recherche active des cas a fait apparaître une incidence maximale de 29 pour 1000 habitants. Dans un village atteint par l'épidémie, une enquête porte à porte a montré que sur 1000 habitants, 34 étaient touchés. Le tableau clinique était dominé par une paraparésie spasmodique à début brutal, souvent associée à une hyperréflexie des membres supérieurs, à une dysarthrie et à une baisse d'acuité visuelle. Chez les patients, le taux de thiocyanate dans les échantillons de sérum et de sang hémolysé atteignait en moyenne 329 μmol/l, chiffre très élevé qui correspond à une intoxication cyanurée chronique. Un taux élevé comparable de 288 μmol/l a été observé chez 22 témoins pris parmi les voisins et les membres de la famille. La recherche de pesticides et de virus s'est révélée négative. Le cyanure provenant de la consommation de manioc était la source la plus probable de thiocyanate. L'épidémie s'est en effet produite dans un secteur où l'aliment de base est le manioc et, du fait de la sécheresse, la seule culture vivrière à ne pas être totalement détruite a été celle de manioc amer. Le séchage du manioc, qui en assure normalement la détoxication mais prend longtemps, n'a pu être mené à bien par suite de la pénurie alimentaire. Selon toute probabilité ou peut égale-

ment mettre en cause une carence du régime, peut-être en acides aminés soufrés. Une maladie épidémique présentant un tableau clinique semblable a été décrite dans une région du Zaïre où le manioc est l'aliment de base. Le mantakassa diffère des cas de neuropathie ataxique tropicale antérieurement signalés en association avec une intoxication cyanurée chronique car le tableau clinique est dominé par le début brutal de la paraparésie spasmodique et la rareté des signes sensoriels. Les résultats des investigations ci-dessus donnent fortement à penser qu'il existe une association entre l'épidémie de paraparésie spasmodique, l'intoxication cyanurée chronique et la consommation de manioc.

REFERENCES

1. OSUNTOKUN, B. O. Cassava diet, chronic cyanide intoxication and neuropathy in the Nigerian Africans. *World review of nutrition and dietetics*, **36**: 141–173 (1981).
2. TROLLI, G. *Résumé des observations réunies, au Kwango, au sujet de deux affections d'origine indéterminée: 1. Paraplégie spastique épidémique. 2. Syndrome œdémateux, cutané et dyschromique.* Brussels, Fonds Reine Elisabeth, 1938.
3. PETTIGREW, A. R. & FELL, G. S. Simplified colorimetric determination of thiocyanate in biological fluids, and its application to the investigation of the toxic amblyopias. *Clinical chemistry*, **18**: 996 (1972).
4. LUNDQUIST, P. ET AL. Method for determining thiocyanate in serum and urine. *Clinical chemistry*, **25**: 678–681 (1979).
5. CRUIKSHANK, E. K. Effects of malnutrition on the central nervous system and the nerves. In: *Handbook of clinical neurology. Vol. 28. Metabolic and deficiency diseases of the nervous system, Part 2.* Amsterdam, Elsevier North-Holland, 1976, pp. 1–41.
6. VILEN, A. F. Contribution à la discussion de la paraplégie spastique épidémique du Kwango. *Annales de la Société Belge de Médecine Tropicale*, **22**: 309–317 (1942).
7. *Weekly epidemiological record*, **57**: 213–214 (1982).
8. MAKENE, W. J. & WILSON, J. Biochemical studies in Tanzanian patients with ataxic tropical neuropathy. *Journal of neurology, neurosurgery, and psychiatry*, **35**: 31–33 (1972).
9. ERMANS, A. M. ET AL. *Role of cassava in the etiology of endemic goitre and cretinism.* Ottawa, International Development Research Centre, 1980.
10. MCMILLAN, D. E. & GEEVARGHESE, P. J. Dietary cyanide and tropical malnutrition diabetes. *Diabetes care*, **2**: 202–208 (1979).
11. BOYLAND, E. & WALKER, S. A. Effect of thiocyanate on nitrosation of amines. *Nature*, **248**: 601–602 (1974).

Bulletin of the World Health Organization, 62 (3): 485–492 (1984)

© World Health Organization 1984

Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 2. Nutritional factors and hydrocyanic acid content of cassava products*

MINISTRY OF HEALTH, MOZAMBIQUE[1]

An outbreak of spastic paraparesis which mostly affected women and children occurred in a northern province of Mozambique in 1981. The epidemic was related to chronic cyanide intoxication associated with a diet consisting almost exclusively of cassava. A prolonged drought in the area had exhausted all food resources except cassava, especially the bitter varieties. A nutritional, toxicological and botanical investigation was carried out in two of the five districts affected. The main findings were that cyanide levels were unusually high in the cassava plant as a consequence of the drought with daily intakes estimated at 15–31.5 mg HCN. Detoxification of the bitter varieties by sun-drying was inadequate because of the general food shortage, and metabolic detoxification was probably reduced owing to the absence of sulfur-containing amino acids in the diet. The raw and dried uncooked cassava was eaten mostly by women and children. The nutritional status of the population, however, was not very poor and symptoms of advanced undernutrition were rarely seen.

An outbreak of spastic paraparesis occurred between May and October 1981 in Nampula Province, northern Mozambique, and involved five districts: Memba, Erati, Nacala, Maiaia and Monapo. The disease was called *mantakassa*, which means paralysis in the local language. The outbreak occurred in a drought-stricken rural area, where the staple food was cassava, and affected mostly women and children. The epidemiological, clinical and laboratory findings suggested that the disease was associated with cyanide intoxication and cassava consumption (see Part 1). Recently a neurological disease associated with chronic cassava intoxication was described in Nigeria (*1*). Mantakassa also shows clinical and epidemiological similarities with lathyrism in India (*2*).

This second part of the study describes the nutritional, toxicological, and botanical investigations that were performed to test the possibility of a food-related cause. These investigations were carried out in September and October 1981 in the districts of Memba and Maiaia. The objective was to study the agricultural and nutritional situation in normal years, to compare this with the changes that occurred in 1981, and to investigate the relationship between cassava consumption and the high thiocyanate levels found in patients with the disease.

The fieldwork was done in the short time available with limited resources. The sample size for the nutrition investigation was small and the results could not, therefore, be subjected to statistical analysis. They do, however, indicate that nutritional and other factors could have played a part in the etiology of the disease.

Ecological description of the area

The area affected by the drought in 1981 can be divided into three distinct ecological and agricultural zones: coastal, intermediate, and interior. The usual rainy season extends from December to March with a mean annual rainfall of about 700 mm along the coast, increasing to 1000 mm in the interior (Fig. 1). Table 1 shows the distribution of crops in the three zones, based on a national agricultural survey carried out in Mozambique in 1961–66 (*3*). This distribution has not changed.

* Part 1 appears on pages 477–484 in this issue of the *Bulletin*. Requests for reprints should be sent to: Secção de Epidemiologia, Direcção Nacional de Medicina Preventiva, Ministério da Saúde, Maputo, Mozambique.

[1] This text was prepared for the Ministry of Health, Mozambique, by *E. Casadei*, Head, Food and Water Hygiene Laboratory, National Directorate of Preventive Medicine, Ministry of Health, Maputo, Mozambique; *P. Jansen*, Botanist, Department of Traditional Medicine, National Directorate of Preventive Medicine; *A. Rodrigues*, Nutritionist, Department of Nutrition, National Directorate of Preventive Medicine; *A. Molin* and *H. Rosling*, former District Medical Officers, Nacala Hospital, Nacala, Mozambique.

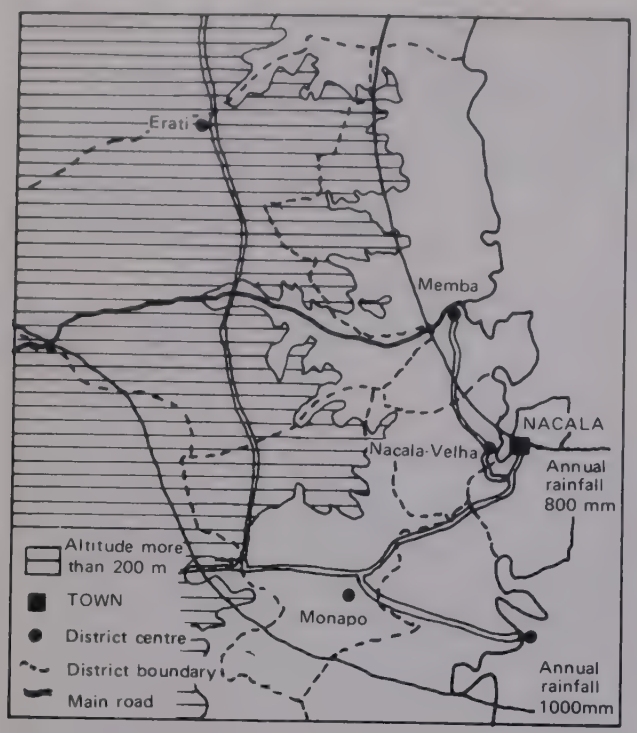


Fig. 1. Altitude and mean annual rainfall in the area affected by the drought.

The dry and sandy *coastal* zone, 10–12 km wide, has a severely limited agricultural capacity. Cereals are rarely grown while cassava occupies 86% of the cultivated area. The predominant cash crops are cashew and sisal. The *interior* zone, which is situated more than 60 km from the coast at an altitude of more than 200 m above sea level, has good agricultural conditions. Although much sorghum is grown, cassava is still the main staple food; cotton is the cash crop. The *intermediate* zone lies between 10 km and 80 km from the coast at an altitude of 50–200 m above sea level. The vegetation consists of secondary deciduous forest with deciduous parkland, open bushland, and spiny savanna (*4*). The number of cashew trees per peasant is among the highest in northern Mozambique; this zone also marks the eastern limit where cotton can be grown. Cassava accounts for 68% of the cultivated area of food crops, with smaller proportions for maize and sorghum. The main protein suppliers in the diet are beans and groundnuts.

The present study refers mainly to the intermediate zone where the disease incidence was highest.

History of cassava in Mozambique

Cassava was probably introduced into Nampula Province, around 1760, from the French colonies in the Indian Ocean (*5*). It became established as the main staple crop along the coast in the period between then and the beginning of the present century. From

Table 1. Distribution of crops in the family sector in the three agricultural zones

Crops	% of area with crops		
	Interior zone	Intermediate zone	Coastal zone
Cassava	59.5	68.5	86.0
Sorghum	18.6	6.6	2.6
Maize	3.3	5.8	1.8
Rice	3.3	0	2.6
Peanuts	10.5	11.0	2.1
Beans	4.2	8.0	3.8
Cotton (as % of the area with traditional crops)	27.2	22.2	0
Cashew (mean number of trees per peasant)	21.9	51.0	45.3

1926 onwards, the Portuguese colonial power introduced a system of forced cultivation of cash crops, like cotton and cashew, which had a negative effect on the subsistence economy. It was during this period that cassava gained prominence as a staple food crop. Its cultivation calendar did not coincide with that for cotton, which resembled that for cereals. The peasants were also obliged to grow cassava for commercial purposes, to help feed the growing number of plantation and urban workers (*6*). In recent years, cash crop production has diminished owing to a combination of factors such as the abolition of forced labour after the country's independence and a breakdown of the commercial network.

As a consequence of the slave trade, cassava became the staple food in many parts of Africa. It yielded more energy per hectare and per man-hour than cereals; and the fact that its edible parts were not needed for sowing and that it was remarkably resistant to drought and pests contributed to its spread. Today, it provides around 40% of the food energy requirements in Africa.

MATERIALS AND METHODS

From August to October 1981 information was obtained on the agricultural and nutritional situation from a variety of sources — local authorities, farmers, teachers, traders, and the general population. Systematic investigations were made of the nutritional situation and samples of cassava products were collected from the following affected areas:

— a communal village, Acordo de Lusaka, 20 km south of Memba;

— the administrative division of Calawane, 40 km northwest of Memba; and

— the administrative division of Muentaze, 20 km south of the port town of Nacala.

The communal village was chosen for study because it had a high incidence of the disease with 28 cases in a population of 815. Seventeen of the affected families were interviewed, as well as sex- and age-matched controls in 17 neighbouring families. Eight affected families in Calawane, which had an incidence of 10.7 per 1000 inhabitants, and 6 affected and 4 neighbouring families in Muentaze were also interviewed.

The nutrition investigation included the collection of information on current agricultural practices, nutritional habits and customs before and during the drought, and socioeconomic conditions. In addition, a review of family food consumption during a 24-hour period and an assessment of nutritional status using anthropometry were carried out in the communal village. Weight-for-height and weight-for-age parameters were used to indicate the degree of "wasting" (*7*) or recent loss of body weight due to reduced food intake; the cut-off points were 75% and 90% for weight-for-height and 60% and 80% for weight-for-age of the Harvard Standards, which correspond to those currently in use by the Ministry of Health in Mozambique.

Various samples of fresh and processed tubers and leaves of cassava were collected for cyanide analysis using uniform collection methods. Fresh samples were placed in a plastic bag immediately after collection, labelled and frozen on the same day, then transported to the laboratory in cold boxes and frozen again immediately on arrival. Dry samples were placed in paper bags until they were analysed, without any special storage or transport conditions. The samples were collected from many different parts of the affected area and were analysed for cyanide content at the National Food and Water Hygiene Laboratory of the Ministry of Health in Maputo, using the alkaline titration method (*8*) and the direct potentiometric method with a cyanide specific electrode (AMEL 201 I).

Samples of domesticated and wild plants consumed by the inhabitants were also collected, identified, and recorded.

RESULTS

Cassava cultivation and diet in normal years

Cassava constitutes about 80% of the staple food in the affected area. Twelve cultivated varieties were identified, two of which were considered to be bitter. It was estimated that the latter accounted for about 30% of the cassava cultivated, but this was higher in some areas. *Gurue*, the more common of the bitter varieties, had been rejected when first introduced in the 1950s, but in recent years was more readily accepted because of its high yield and resistance to drought and attacks from wild animals.

The entire cassava crop is harvested and replanted in August. The fresh cassava is cut into 20-cm-long pieces, sun-dried for 2–3 weeks, and then stored for up to 12 months. The population believes that this long drying period is necessary to make the cassava safe for consumption. Fig. 2 shows that families always reserved some of the previous year's dried cassava to cover the current harvest and drying period.

In these areas the population uses only the sun-drying method of detoxification for bitter cassava; this is eaten uncooked or boiled or, most commonly, after pounding into flour which is mixed with boiling water to make a thick paste called *chima*. The fresh sweet varieties are eaten raw or boiled; they are also dried and processed as for the bitter varieties. Cooked cassava is usually eaten with a relish made from the leaves of the sweet cassava, pumpkin or sweet potato plants, beans, groundnuts, dried fish, tomatoes or coconut depending on availability. People normally eat two meals per day; one normally contains a cereal when this is available in the period after the cereal harvest. Meat and game are rare in the diet; pork is not eaten for religious reasons. Various fruits are eaten but the cashew apple is used to prepare an alcoholic drink. Cashew nuts are roasted and seldom used in cooked dishes.

Effect of the drought on the harvest

The rainy season normally extends from December to March, but in 1979–80 the rains failed and in 1980–81 the area had the lowest rainfall ever remembered or recorded. Fig. 3 shows the low flow of

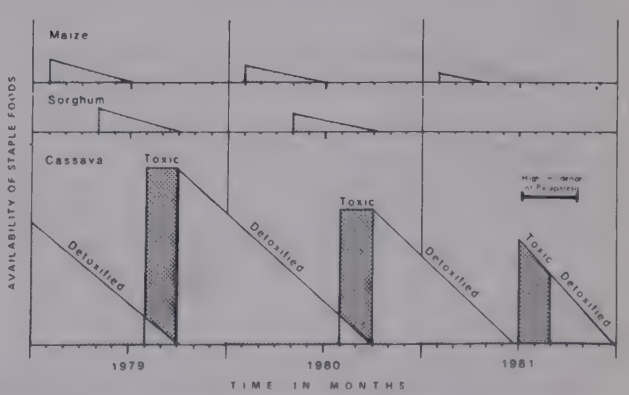


Fig. 2. Availability of staple foods (maize, sorghum, and cassava) from 1979 to 1981 in the area affected by drought.

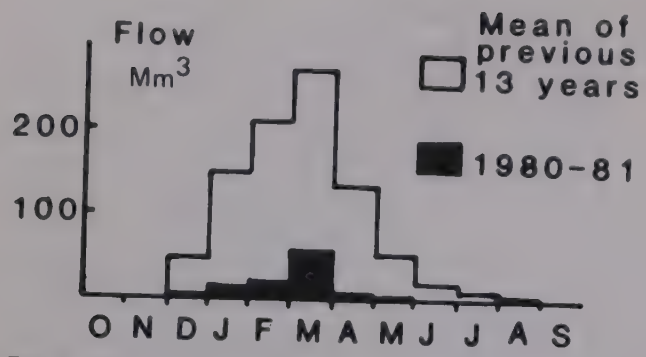


Fig. 3. Flow of the Mecuburi river in the affected area in 1980–81, compared with mean values during the previous 13 years (abscissa shows months from October (O) 1980 till September (S) 1981).

the Mecuburi, the principal river in the most affected area in 1981, which was due to the severity of the drought.

Fig. 2 shows the period of availability after harvesting of the three main staple food crops (maize, sorghum and cassava) from January 1979 to October 1981. Two important points should be noted: firstly, in 1981 cassava was harvested as early as June, while normally it is harvested in August; and second, in the middle of 1981, only the freshly harvested, toxic cassava was available, whereas in 1979 and 1980 some of the previous year's harvest lasted until September.

The production of cereals and legumes was very low or completely lost owing to the drought. Sweet varieties of cassava also suffered severe losses but the bitter varieties produced a reasonable harvest. As a result of the poor harvests, edible wild plants, 110 of which were collected for identification, became an important constituent of the diet.

By the end of June 1981 all normal means of procuring food were exhausted and it became necessary to harvest the cassava. People complained that the surviving sweet cassava had turned bitter, and that the bitter varieties were more bitter than usual. The fresh tubers were cut into 1×1 cm pieces and/or pounded and dried for 1–2 days only. As other foods were scarce, the population began to consume this inadequately dried cassava.

Because the cashew crop also had failed and work was difficult to obtain, ready cash was in short supply. Thus, even when food was occasionally available, some families did not have the means to buy it.

Diet during the drought

Table 2 presents the results of the 24-hour recall on food consumption, which was carried out in the communal village. The low economic status of the affected families is demonstrated by their failure to join the food Consumer Cooperative which required a monetary contribution, and by the fact that these people depended on gifts of food from family and friends. Some affected families also ate fresh bitter cassava and augmented the quantity of flour with pounded, dried cassava peelings. This latter practice was introduced only in 1981. Most families ate twice a day but some, including children, sometimes ate only once a day. In Calawane and Muentaze, the dietary situation was similar, the only exceptions being one family that ate maize and two others that ate dried fish and beans, in Muentaze.

Table 2. Results of a 24-hour recall of food consumption by affected and control families in Acordo de Lusaka, Memba District, in October 1981

	No. of families with positive response	
	Affected families ($n = 17$)	Control families ($n = 17$)
Ate "chima" (made from dried bitter cassava)[a]	17	17
Ate dried, uncooked cassava[a]	16	6
Ate cereals	1	1
Ate beans	1	2
Ate relish (containing cassava leaves)[a]	13	12
Ate fish or meat	0	6
Ate wild plants (in the last month)	1	6
Member of the food Consumer Cooperative	0	6
Depended on gifts of food	6	1

[a] Median estimated drying time of cassava was 7 days for both the affected families and the control families.

Almost all the affected families recalled having acute symptoms 4–6 hours after ingestion of meals containing cassava from the 1981 harvest. However, at the time of the investigation the incidence of acute intoxication had decreased.

Nutritional status

Table 3 shows the results of the investigation of nutritional status of families in Acordo de Lusaka. There is little difference between the status of the affected families and the controls.

Toxicology

Table 4 shows the hydrocyanic acid (HCN) content of the cassava samples using the alkaline titration method. Comparison of this method with the direct

Table 3. Nutritional status in Acordo de Lusaka, Memba District, in October 1981

	No. of persons or children	Parameter[a]	Percentage undernourished	
			Moderate	Severe
Affected families	65	W/H	49	12
Control families	37	W/H	38	11
Village children:				
0–1 year old	29	W/A	45	14
1–6 years old	121	W/H	32	2

[a] W/H = weight for height; W/A = weight for age.

Table 4. Hydrocyanic acid (HCN) content of cassava samples from the affected area

Type of cassava sample	No. of samples	HCN (mg/kg)		
		Mean value	Standard deviation	Range
Fresh leaves:				
bitter varieties	6	377	110	224–469
sweet varieties	14	347	178	71–495
Fresh roots:				
bitter varieties	15	327	153	186–446
sweet varieties	20	138	55	54–235
Dried roots:				
bitter varieties	12	95	47	64–178
sweet varieties	3	46	9	35–54
Flour	5	40	9	26–43
Cooked cassava	10	38	22	19–87

potentiometric method gave a maximum variation of 10%. HCN values were highest for the fresh leaves, with no significant difference between bitter and sweet varieties; the mean HCN value was higher in fresh roots of the bitter than sweet varieties ($P < 0.01$) but there was considerable overlap. Progressively lower mean values were found in the dried samples, the reduction in cyanide content being 70% for the bitter varieties and 67% for the sweet varieties. The greatest range of variation in HCN values occurred in the fresh bitter roots.

The relationship between the cyanide content, the length of drying time, and humidity showed that the cyanide levels appeared to decrease rapidly with drying (within one week) and then remained stationary. Even with prolonged drying of two months and over, the HCN levels were still high: 85 mg/kg for bitter cassava and 35 mg/kg for sweet cassava.

DISCUSSION

Historical factors

The problem encountered in 1981 had its real beginnings in the colonial period when cassava was first introduced into the area. Virtual dependence on this one crop was created at the expense of subsistence production of cereals, legumes, etc. This, in turn, created a dependence on commercially available food which consequently generated the need to earn money through sale of cash crops or manual labour for wages. In recent years the commercially available food has diminished for several reasons such as a decline in cash crop production because of unfavourable weather conditions, the abolition of forced cash crop production, and a breakdown in the commercial network. Owing to the shortage of cash and of food on sale, the dietary supply of protein-rich foods containing the sulfur necessary for metabolic detoxification of cyanide was probably reduced.

Pressure of demand from the coastal and urban centres for food from the interior continued during the drought, thereby encouraging the rural population to sell the dried cassava and other food that they had.

Agricultural potential

The non-coastal areas most affected by the 1981 drought and outbreak of mantakassa had always had a limited agricultural potential and a relatively high population density, but this did not prevent them from exporting food to other areas. The coastal zone, in comparison, could not produce sufficient food and imported it from the interior, although fishing provided a useful supplement. The coastal zone was least affected by the outbreak, possibly because of the continued supply of fish which provided both the sulfur-containing amino acids needed for metabolic detoxification of cyanide, and the means for exchanging food with the interior. The urban populations, on the other hand, ate mainly dried cassava from the interior and were therefore prone to the risk of intoxication.

Detoxification

Unlike other cassava-producing areas where the cassava is left in the ground and harvested as required, the crop in Nampula is harvested over a short period which is followed by extensive drying and storage periods. The practice in Nampula probably

evolved both to fit in with the dates for cotton cultivation during the year and to allow sufficient time for the cassava to dry out adequately, especially in the case of the bitter varieties. As sun-drying is the only method of detoxification used, the drying period is particularly important.

In a review of traditional detoxification methods, Coursey (*9*) states that "sun-drying can only be applied to cassava containing small amounts of glycoside". It is therefore surprising that in an area where bitter cassava had for a long time been cultivated, other more efficient detoxification methods (such as soaking) were not practised. In other areas of Mozambique, however, methods such as soaking and grating are used (*10*). Soaking has been shown to reduce the glycoside content by about 90%, while sun-drying and boiling were less effective (*11*). Also, a study among six ethnic groups in Zaire (*12*) discussed the importance of detoxification methods in relation to cyanide intake; a direct relationship was found between the incidence of goitre attributed to a high cyanide intake linked with a low iodine supply, and the methods of preparation used. Soaking, again, gave more favourable results.

Diet and nutritional status

Before the drought, the diet in the affected area was probably well balanced in terms of energy and nutrient requirements. The drought then caused a severe reduction and imbalance in the diet, which mostly affected the women of reproductive age and children who probably consumed the raw and dried uncooked cassava, which are higher in cyanogenic glycosides, in the course of collection and preparation. The children were affected because they often accompany women in their tasks. In contrast, the men normally ate well-cooked cassava. Lactating women who had a high incidence of the disease may also have eaten more raw cassava which was believed to stimulate lactation.

Studies in a cassava-eating population in Zaire (*13*) have shown that the thiocyanate level in breast milk, although low, was not negligible but breast-fed babies did not present with the disease. The protein in the breast milk could have exerted a protective effect; in older children, however, the introduction of other foods possibly raised their cyanide intake. Vanderpas et al. (*14*) reported that when cassava juice was introduced as a first weaning food, the serum thiocyanate levels rose.

Table 2 shows that a larger proportion of the affected families ate dried uncooked cassava which could have been an important factor in raising the cyanide intake. The similarity in nutritional status between the affected and control groups confirms the general lack of food in the area. Protein-rich foods, particularly, were insufficient and could have resulted in diminished metabolic detoxification. In spite of the general food shortage, the usual symptoms of advanced undernutrition such as growth failure, oedema, wasted muscles, and apathy were rarely seen. However, many cases of kwashiorkor did appear in February 1982 after the rains failed again at the end of 1981.

The high serum thiocyanate levels in both patients and controls must have been due to chronic cyanide intoxication. Factors in the diet such as high cyanogenic glycoside levels in the cassava plant and inadequate detoxification of the cassava, as well as insufficient metabolic detoxification due to a dietary deficiency of sulfur-containing amino acids, may have triggered the disease.

As none of the wild plants identified is known to be neurotoxic, it is unlikely that they played a role in the etiology of the disease. They did provide some food during the critical period, but the drought considerably reduced this source too.

Toxic levels in cassava products

The cyanide levels found in the cassava samples in this investigation are high when compared with those found by other authors (*15*, *16*). In this study, 55% of the sweet fresh roots were extremely toxic and the remainder moderately so, which may have been due to the drought conditions increasing the cyanogenic glycoside content (*17*). The mean values were higher in the roots of the bitter varieties. In general, the dried samples had lower cyanide levels, but even with two months of drying some values were still high. This finding confirms the opinion of Coursey (*9*), mentioned above.

To relate the values found in the samples to the amount of cyanide ingested, the dietary habits of the population must be considered. The highest values were found in fresh leaves but these are normally eaten cooked which probably detoxifies much of the cyanide. If it is assumed that 90% of the diet was composed of cassava, then with a daily adult energy intake of 7.53 megajoules (MJ) (1800 $kcal_{th}$) the quantity of cassava consumed would have been in the range of 500–700 g per day. Most of the dried bitter cassava samples had a cyanide content of 80–100 mg/kg, while for the cooked samples the range was 30–45 mg/kg. A calculation based on a diet containing dried bitter cassava gives an approximate daily intake of 15–31.5 mg HCN. These levels of intake may be compared with the adult lethal dose of cyanide of 50 mg and could explain the frequent acute reactions after meals. Over a period of time, the consumption of such quantities could result in chronic cyanide intoxication, which was found in both patients and controls. Given the dependence of this population on cassava, and the proneness of the area to drought, people

possibly consume sufficient cassava to result in chronic cyanide intoxication even under more normal conditions. They could therefore be at risk from other diseases reported in association with chronic cyanide intoxication such as chronic neurological disease (*1*), endemic goitre and cretinism (*12*), and diabetes (*18*).

Cassava and economic development

As one of the cheapest sources of food energy available, cassava plays a very important role in countries such as Mozambique, where peasant populations are subjected to unreliable climatic conditions and need security in their food supplies; owing to its hardiness and natural resistance, it often provides this security. The solution to the problem of cyanide intoxication, however, is not the prohibition of cassava but a careful study of the agricultural situation so that other drought-resistant crops including cereals and legumes, and varieties of cassava with lower cyanogenic glycoside levels may be introduced. At the same time, efforts to promote economic and social development in the areas prone to such disasters offer the only long-term solution.

CONCLUSIONS

The following evidence links the chronic cyanide intoxication found in patients to cassava consumption:

— raised levels of cyanogenic glycoside levels in the cassava plant, including sweet varieties, which were due to the drought;

— higher levels than usual of cyanide in processed cassava products because of inadequate detoxification;

— the almost total dependence on cassava in the diet during the drought period, and reduced metabolic detoxification of cyanide because of the lack of protein-rich foods.

ACKNOWLEDGEMENTS

The help of the following in this collective work is acknowledged: community leaders, administrative and health workers at the district level, the provincial health administration (especially Mr Ilidio Manjate and Mr Constantino Cuamba of the Nutrition Section and, in Maputo, Dr Jorge Cabral and Dr Ana Novoa of the National Directorate of Preventive Medicine), staff of the National Food and Water Hygiene Laboratory, and members of the Department of History at Eduardo Mondlane University.

RÉSUMÉ

LE MANTAKASSA: ÉPIDÉMIE DE PARAPARÉSIE SPASMODIQUE ASSOCIÉ À UNE INTOXICATION CYANURÉE CHRONIQUE, DANS UNE RÉGION DU MOZAMBIQUE OÙ LE MANIOC EST L'ALIMENT DE BASE. 2. FACTEURS NUTRITIONNELS ET TENEUR EN ACIDE CYANHYDRIQUE DES PRODUITS DU MANIOC

Une épidémie de paraparésie spasmodique ou *mantakassa* (de son nom local) a éclaté en 1981 dans cinq districts d'une province septentrionale du Mozambique. C'est dans une région agricole assez pauvre et touchée par la sécheresse que l'incidence la plus forte a été observée. Les principales victimes étaient les femmes et les enfants. Etant donné la rareté des denrées alimentaires, la population s'est presque exclusivement nourrie de manioc, de variétés amères en particulier. Parmi les douze variétés de manioc cultivées dans la région, deux seulement sont amères à proprement parler; mais par suite de la sécheresse toutes les variétés se sont révélées plus amères qu'à l'ordinaire. Le manioc se détoxique normalement par séchage au soleil et stockage pendant une durée qui va jusqu'à un an. Mais en 1981, à cause de la pénurie alimentaire, le manioc a été récolté de bonne heure, concassé et séché au soleil un ou deux jours seulement avant d'être consommé.

L'existence d'un lien possible entre la consommation de manioc et une intoxication cyanurée a été explorée dans les zones les plus touchées au moyen d'investigations portant sur la nutrition, la toxicologie et la botanique. Sur le plan de la nutrition, les familles touchées et les témoins ont été étudiés sous l'angle des facteurs agricoles, économiques et sociaux et des habitudes alimentaires avant et pendant la sécheresse, notamment la nature de la consommation journalière. L'analyse d'échantillons de produits du manioc a révélé, tant dans les produits séchés que dans les produits frais, une teneur anormalement élevée en hétéroside, qui libère par hydrolyse de l'acide cyanhydrique. Le taux de cyanure résiduel dans les échantillons déshydratés atteignait respectivement 85 et 35 mg/kg dans les variétés amères et douces.

La ration de cyanure a été estimée à 15–31,5 mg par personne et par jour. De plus, il s'est produit une pénurie d'aliments riches en protéines contenant des acides aminés soufrés qui auraient pu renforcer la détoxification métabolique du cyanure. Les symptômes d'une intoxication aiguë ont été ressentis 4 à 6 heures après les repas comportant du manioc de la récolte de 1981. Plus de 100 variétés de plantes sauvages consommées ont été récoltées et identifiées; aucune

n'a de propriétés neurotoxiques connues. Les familles économiquement défavorisées et qui ont consommé du manioc séché cru et mangé moins de viande ou de poisson étaient relativement plus nombreuses parmi les familles atteintes que parmi les témoins. Les femmes et les enfants ont probablement consommé davantage de manioc séché non cuit, car ce sont eux qui sont chargés de la récolte et de la préparation. Aucune différence importante n'a été notée entre les groupes atteints et les groupes témoins en ce qui concerne l'état nutritionnel; des signes de dénutrition grave ont rarement été observés. Le lien entre l'intoxication cyanurée chronique et la consommation de manioc ressort de diverses observations, parmi lesquelles il convient de citer l'absorption anormalement élevée de cyanure pendant la sécheresse, par suite de la teneur accrue de la plante en hétéroside libérant de l'acide cyanhydrique, et le manque d'acides aminés soufrés dans le régime alimentaire.

REFERENCES

1. Osuntokun, B. O. Cassava, diet, chronic cyanide intoxication and neuropathy in Nigerian Africans. *World review of nutrition and dietetics*, **36**: 141–173 (1981).
2. Hartman, C. P. et al. A study on *Lathyrus sativus*. *Indian Journal of nutrition and dietetics*, **11**: 178–191 (1974).
3. Carvalho, M. de [*Traditional agriculture in Mozambique*. The geographical distribution of the cultivations and their impact on the environment.] Lourenço Marques, Missão de Inquérito Agrícola de Moçambique, 1969, pp. 1–67 (in Portuguese).
4. Gomes, P. J. & Grandvaux Barbosa, L. A. [The vegetation.] In: [*Sketch of an ecological and agricultural survey in Mozambique.*] Lourenço Marques, Centro de Investigação Científica Algodoeira. *Memórias e Trabalhos*, No. 23, 1955, pp. 67–224 (in Portuguese).
5. Serra, C. [*Cassava: what it is and how it appeared in Africa and in Mozambique.*] *Tempo*, (619): 51 (1982) (in Portuguese).
6. Centro de Estudos Africanos. [*The transformation of agriculture at the family level in the province of Nampula.*] *C.E.A. Relatório*, No. 80/3, Maputo, Eduardo Mondlane University, 1980 (in Portuguese).
7. Waterlow, J. C. Classification and definition of protein-energy malnutrition. In: Beaton, G. H. & Bengoa, J. M., ed., *Nutrition in preventive medicine*, Geneva, World Health Organization, 1976, pp. 530–555.
8. Horwitz, W. ed., *Official methods of analysis of the Association of Official Chemists*. Washington, Association of Official Analytical Chemists, 1980, p. 433.
9. Coursey, D. G. Cassava as food: toxicology and technology. In: *Chronic cassava toxicity*, Ottawa, International Development Research Centre, 1973, pp. 27–36.
10. Mota, T. P. [*The cassava flour of Mozambique.*] *Agronomia Moçambicana*, **8**: 47 (1974) (in Portuguese).
11. Bourdoux, P. et al. Cassava products: HCN content and detoxification processes. In: *Nutritional factors involved in the goitrogenic action of cassava*, Ottawa, International Development Research Centre, 1982, pp. 51–58.
12. Simons-Gérard et al. Foods consumed and endemic goitre in the Ubangi. In: *The role of cassava in the etiology of endemic goitre and cretinism*. Ottawa, International Development Research Centre, 1980, pp. 69–80.
13. Delange, P. et al. Effects of thiocyanate during pregnancy and lactation on thyroid function in infants. In: *Role of cassava in the etiology of endemic goitre and cretinism*, Ottawa, International Development Research Centre, 1980, pp. 121–126.
14. Vanderpas, J. et al. Breast feeding, thiocyanate metabolism and thyroid function in young infants in severe endemic goitre. In: *Nutritional factors involved in the goitrogenic action of cassava*, Ottawa, International Development Research Centre, 1982, pp. 59–64.
15. Bolhuis, G. C. The toxicity of cassava roots. *Journal of agricultural science*, **2**: 176–185 (1954).
16. Oke, O. I. The role of HCN in nutrition. *World review of nutrition and dietetics*, **11**: 170–198 (1969).
17. De Bruijn, G. H. The cyanogenic character of cassava. In: *Chronic cassava toxicity*. Ottawa, International Development Research Centre, 1973, pp. 43–48.
18. McMillan, D. E. & Geevarghese, J. Dietary cyanide and tropical malnutrition diabetes. *Diabetes care*, **2**: 202–208 (1979).

THE LANCET, NOVEMBER 30, 1985

ASSOCIATION OF HIGH CYANIDE AND LOW SULPHUR INTAKE IN CASSAVA-INDUCED SPASTIC PARAPARESIS

JULIE CLIFF
PER LUNDQVIST
JOHANNES MÅRTENSSON
HANS ROSLING
BO SÖRBO

Epidemiological Section, Ministry of Health, Mozambique; Department of Clinical Chemistry, Linköping University; and International Child Health Unit and Department of Internal Medicine, Uppsala University, Sweden

Summary Urinary excretion of sulphur compounds was studied in children from a population in Mozambique that had been affected, during a drought, by an epidemic of spastic paraparesis attributed to cyanide exposure from cassava. The children had increased thiocyanate and decreased inorganic sulphate excretion, indicating high cyanide and low sulphur-containing amino-acid intake. Children from a neighbouring cassava-eating area, where no cases of spastic paraparesis had occurred, had lower thiocyanate excretion but higher inorganic sulphate excretion. These results support the hypothesis that the epidemic was due to the combined effects of high dietary cyanide exposure and sulphur deficiency.

Introduction

NUTRITIONAL neuropathies occur in many malnourished populations in various tropical countries.[1] Exposure to dietary cyanide from cassava has been implicated in the aetiology of certain nutritional neuropathies in Africa.[2-4] Cassava roots, which are an important staple crop in many tropical countries,[5] contain cyanogenic glycosides which are removed by processing based on soaking, roasting, or sun-drying, before consumption. When the roots are eaten without processing, often as the result of food shortage during a drought, the dietary intake of cyanide can be high. After ingestion cyanide is detoxified by conversion to thiocyanate,[6] the enzymatic pathways depending on sulphur-containing aminoacids. A deficiency of sulphur aminoacids may therefore impair cyanide detoxification capacity;[7] a combination of high cyanide exposure and deficiency of sulphur aminoacids has been suggested as the cause of an ataxic neuropathy occurring among cassava-eaters in Nigeria.[8]

An outbreak of another form of nutritional neuropathy, acute spastic paraparesis, has been reported from a drought-striken area of Mozambique.[9] The epidemic occurred during the cassava harvest in 1981 when toxic cassava varieties with very high cyanide content were the only food available and when, due to lack of other food, the roots were eaten without the usual sun-drying for removal of cyanide.[10] Blood thiocyanate levels in patients with spastic paraparesis were very high,[9] indicating considerable cyanide exposure. During the epidemic the population also had a low dietary intake of protein, although frank malnutrition was rare in

patients with paraparesis.[10] Inadequate supply of sulphur aminoacids for cyanide detoxification therefore seemed likely, although no laboratory investigations were done to support this hypothesis.[11]

We have now studied the urinary excretion of sulphur compounds in the affected population during the first two harvest periods following the epidemic of spastic paraparesis. Although no new cases of paraparesis were detected during these two harvests, the dietary circumstances were similar but less severe than in 1981. Schoolchildren were chosen for the study because the epidemic mainly affected children of that age. Urinary excretion of total sulphur and inorganic sulphate was determined as a measure of dietary intake of sulphur aminoacids;[12] urinary thiocyanate[13] was measured as an indicator of cyanide exposure. The results were compared with those of a neighbouring cassava-eating population that had not been affected by the paraparesis epidemic and of urban non-cassava eating children in Mozambique and Sweden.

Subjects, Materials, and Methods

The study was done in Nampula Province in northern Mozambique, where the population consists mainly of peasants who grow cassava, maize, sorghum, and beans. In 1981 the north-eastern part of the province suffered a severe drought; the one crop to survive was the most toxic variety of cassava and, due to lack of food during the harvest period, the roots were eaten after only a few days of sun-drying. Shortly afterwards cases of paraparesis appeared in the area; a field survey during August to October, 1981 revealed 1102 cases of spastic paraparesis.[9] We selected the communal village of Acordo de Lusaka in the Memba district for this follow-up study because of its location in the centre of the epidemic. The first part of the study was done during the 1982 harvest when dietary circumstances was slightly better than in 1981, partly due to regular distribution of limited amounts of relief aid. In spite of this, there was a food shortage and the people had to eat toxic cassava roots after only 1–2 weeks of sun-drying. Urine was collected from 30 apparently healthy children aged 8·1±0·3 years (mean±SEM) in the village school. Reference values for Swedish children were obtained from 17 healthy children aged 8·6±0·1 years.

The second part of the study was carried out during the 1983 harvest when the nutritional situation had improved further but was still unsatisfactory—cassava roots were sun-dried for about 1 month before consumption compared with 2 months in normal years. Urine was collected from 31 children in the village school aged 9·0±0·3 years, and from 30 schoolchildren aged 9·3±0·3 years in the small administrative centre of the Memba district. In this semi-urban population, cassava consumption was also high, but there were no cases of paraparesis during the 1981 epidemic. Urine was also collected from 28 children aged 7·1±0·1 years in the city of Nampula who ate virtually no cassava. Since it was impossible to obtain 24 h urine collections, excretion values of the compounds studied were expressed as creatinine ratios. All urine specimens were collected between 0900 h and 1100 h with thymol-isopropanol as a preservative, and specimens were frozen within 6 h of collection. They were later transported to Sweden for analysis. Creatinine was determined by a conventional alkaline picrate method and determinations of thiocyanate, inorganic sulphate, ester sulphate, and total sulphur were done as previously reported.[13-15] Student's *t* test was used for statistical comparisons.

Results

In 1982, schoolchildren in the area where the epidemic had occurred had very high levels of urine thiocyanate by

TABLE I—URINARY EXCRETION IN 1982 IN APPARENTLY HEALTHY CASSAVA-EATING CHILDREN IN THE AFFECTED AREA AND SWEDISH CONTROLS

Compounds	Cassava and paraparesis (n=30)	Controls (n=17)
Thiocyanate	125±15*	3·4±0·6
Inorganic sulphate	0·41±0·04*	2·16±0·14
Ester sulphate	0·38±0·02*†	0·19±0·01
Total sulphur	1·17±0·05*	2·56±0·16

Results are presented as mean±SEM and given as mol/mol creatinine, except for thiocyanate which is mmol/mol.
*Significantly different from controls ($p<0·05$).
†n=24.

comparison with Swedish controls (table I). Excretion of inorganic sulphate and total sulphur was significantly lower than in the controls whereas excretion of ester sulphate was significantly higher. In 1983, two years after the epidemic (table II) thiocyanate excretion in the study group had decreased by comparison with 1982 (table I) whereas excretion of inorganic sulphate and total sulphur had increased and excretion of ester sulphate was unchanged. Thiocyanate excretion was higher in the area previously affected by paraparesis by comparison with the neighbouring area not affected by the epidemic, and was very high by comparison with the urban area. Excretion of inorganic sulphate and total sulphur (table II) was significantly lower in the area affected by paraparesis by comparison with the other two areas which did not differ in this respect. Excretion of ester sulphate was similar in all three groups.

Discussion

The high thiocyanate excretion found in cassava-eating children from the rural area affected by spastic paraparesis indicates that cyanide intake was high during the cassava harvest the first year after the epidemic; it was slightly lower the following year. The results also suggest a high dietary cyanide intake in cassava-eating children from the neighbouring semi-urban area not affected by the epidemic and, as expected, a very low cyanide intake in urban children.

The low excretion of inorganic sulphate and total sulphur in the population that had been affected by paraparesis indicated a low intake of sulphur aminoacids; The sulphur deficiency was more severe during the first study period. Cassava is a rich energy source in the form of carbohydrate but contains little protein and has a low content of sulphur aminoacids.[16] Moreover, during the drought and in the following years, the population studied was also short of protein-rich supplementary foods such as beans and fish.

The high excretion of ester sulphate in children from Mozambique is of interest as it has been reported that in

children with kwashiorkor the excretion is low.[17] Since the ester sulphate fraction in urine mainly consists of sulphate conjugates of compounds produced by the action of gut bacteria on aromatic aminoacids,[18] these discordant results may be due to different intestinal flora.

The results shown in table II indicate a lower sulphur intake in the rural area affected by paraparesis than in the semi-urban area where, despite high cassava consumption, paraparesis was not observed. This supports an aetiological role for sulphur deficiency in the epidemic of spastic paraparesis. Sulphate excretion in children from the affected area was lower than that in rural children in Kenya with diets deficient in sulphur aminoacids.[19] Our findings also confirm

TABLE II—URINARY EXCRETION IN 1983 IN APPARENTLY HEALTHY CHILDREN FROM AREAS DIFFERING WITH RESPECT TO CASSAVA-CONSUMPTION AND OCCURRENCE OF PARAPARESIS

Compound	Cassava and paraparesis (n=31)	Cassava and no paraparesis (n=30)	No cassava and no paraparesis (n=28)
Thiocyanate	83·9±9·3*†	52·0±12·6*	8·9±2·7
Inorganic sulphate	0·73±0·08*†	1·34±0·17	1·36±0·10
Ester sulphate	0·34±0·03	0·42±0·09	0·27±0·03
Total sulphur	1·42±0·09*†	2·42±0·36*	1·97±0·12

Results are presented as in table I.
*Significantly different from the no cassava group ($p<0·05$).
†Significantly different from the cassava/no paraparesis group ($p<0·05$).

earlier reports[12,19,20] that sulphate excretion is a better indicator of low dietary intake of sulphur aminoacids than is total sulphur excretion.

Although plasma and urine levels of sulphur aminoacids have been used as measures of dietary intake of these compounds,[8] determination of inorganic sulphate is preferable because of its simplicity and cheapness.[15] Sulphate is also considerably more stable in urine than are sulphur aminoacids.

Our results support the hypothesis that the epidemic of spastic paraparesis was caused by the combination of a high intake of cyanide and a low intake of sulphur aminoacids. It has been suggested[8] that sulphur deficiency impairs detoxification of the high cyanide load and that nervous system damage is caused by cyanide per se. Another possible mechanism is that preferential use of the limited amounts of sulphur aminoacids for cyanide detoxification leads to impaired synthesis of sulphur compounds essential for neural function.[21]

Quite apart from the underlying mechanism, it is important to prevent spastic paraparesis in drought-prone cassava-eating areas by making available supplementary foods rich in sulphur aminoacids such as beans and fish,[16] and to promote better processing methods for cassava.[5] It has to be remembered that people in such areas have good reason to continue to grow toxic varieties of cassava as these have been shown to constitute their best protection against famine.

We thank the Ministry of Health in Mozambique for help with this study. The work was supported by grant 83/204 from the Swedish Agency for Research Cooperation with Developing Countries and by Grant 03X-05644 from the Swedish Medical Research Council.

Correspondence should be addressed to H. R., International Child Health Unit, University Hospital, S-751 85 Uppsala, Sweden.

REFERENCES

1. Cruickshank EK. Effects of malnutrition on the central nervous system and the nerves. In: Handbook of clinical neurology vol 28, part 2. Metabolic and deficiency diseases of the nervous system. Amsterdam: Elsevier North-Holland, 1976: 1–41.
2. Osuntokun BO. An ataxic neuropathy in Nigeria: A clinical, biochemical and electrophysiological study. *Brain* 1968; **91:** 215–48.
3. Osuntokun BO. Cassava diet, chronic cyanide intoxication and neuropathy in the Nigerian Africans. *World Rev Nutr Diet* 1981; **36:** 141–73.
4. Makene WJ, Wilson J. Biochemical studies in Tanzanian patients with ataxic tropical neuropathy. *J Neurol Neurosurg Psychiatry* 1972; **35:** 31–33.
5. Cock JH. Cassava: A basic energy source in the tropics. *Science* 1982; **218:** 755–62.
6. Sörbo B. Rhodanese and mercaptopyruvate transulfurase. In: Greenberg DM, ed. Metabolism of sulfur compounds. Metabolic pathways vol VII. New York: Academic Press, 1975: 433–56.
7. Wilson J. Cyanide in human disease: A review of clinical and laboratory evidence. *Fund Applied Toxicol* 1983; **3:** 397–99.
8. Osuntokun BO, Durowoju JE, McFarlane H, Wilson J. Plasma aminoacids in the Nigerian nutritional ataxic neuropathy. *Br Med J* 1968; **3:** 647–49.
9. Ministry of Health, Mozambique. Mantakassa: An epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 1. Epidemiology and clinical and laboratory findings in patients. *Bull WHO* 1984; **62:** 477–84.
10. Ministry of Health, Mozambique. Mantakassa: An epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 2. Nutritional factors and hydrocyanic acid content of cassava products. *Bull WHO* 1984; **62:** 485–92.
11. Editorial. Epidemic spastic paraparesis. *Lancet* 1984; ii: 904–05.
12. Sabry ZI, Shadarevian SB, Cowan JW, Cambell JA. Relationship of dietary intake of sulphur amino-acids to urinary excretion of inorganic sulphate in man. *Nature* 1965; **206:** 931–33.
13. Lundquist P, Mårtensson J, Sörbo B, Öhman S. Method for determining thiocyanate in serum and urine. *Clin Chem* 1979; **25:** 678–81.
14. Lundquist P, Mårtensson J, Sörbo B, Öhman S. Adsorption of thiocyanate by anion-exchange resins and its analytical application. *Clin Chem* 1983; **29:** 403.
15. Lundquist P, Mårtensson J, Sörbo B, Öhman S. Turbidimetry of inorganic sulfate, ester sulfate and total sulfur in urine. *Clin Chem* 1980; **26:** 1178–81.
16. Cameron M, Hofvander Y. Manual on feeding infants and young children. Oxford: Oxford University Press, 1983.
17. Ittyerah TR. Urinary excretion of sulfate in kwashiorkor. *Clin Chim Acta* 1969; **25:** 365–69.
18. Boström H. Sulfate conjugation and conjugated sulfates. *Scand J Clin Lab Invest* 1965; **17** (suppl 86): 33–52.
19. Simmons WK. Use of the inorganic sulfate/creatinine ratio in field studies. *Am J Clin Nutr* 1973; **26:** 72–76.
20. Folin O. Laws governing the chemical composition of urine. *Am J Physiol* 1905; **13:** 66–115.
21. Cavallini D, Gaull GE, Zappia V, eds. Natural sulfur compounds. Novel biochemical and structural aspects. New York: Plenum, 1980.

[Reprinted from CLINICAL CHEMISTRY, 31, 591 (1985).]
Copyright 1985 by the American Association of Clinical Chemistry and reprinted by permission of the copyright owner.

Determination of Cyanide in Whole Blood, Erythrocytes, and Plasma

Per Lundquist,[1] Hans Rosling,[2] and Bo Sörbo[1,3]

We describe a method for determination of cyanide in whole blood, erythrocytes, and plasma after stabilization of cyanide by addition of silver ions. The cyanide is then transferred from the acidified sample, by aeration, into sodium hydroxide and quantified by the König reaction, with sodium hypochlorite as the chlorinating agent. A rapid loss of measurable cyanide found when cyanide was added to plasma in the absence of silver ions was attributed to a reaction with serum albumin. Cyanide added to whole blood was bound to a saturable component in erythrocytes, which we identified as methemoglobin.

Additional Keyphrases: *thiocyanate · toxicology · reference interval · smoking · binding by methemoglobin*

Occupational exposure to cyanide may occur in connection with electroplating, metal refining, case hardening of steels, and other industrial processes (*1, 2*). The presence of hydrogen cyanide in fire gas (*3*) is a threat to fire fighters as well as fire victims, and its presence in tobacco smoke represents an important health problem (*4*). Cyanide has also been implicated as a causative agent in certain diseases (*5*), and cyanide poisoning is occasionally used as a mode of suicide (*6*). Furthermore, the recent introduction of sodium nitroprusside as a hypotensive agent has produced cases of iatrogenic cyanide poisoning (*7, 8*). Finally, a cause of chronic cyanide poisoning on a large scale in certain tropical areas is the consumption of cassava, a food stuff containing a cyanogenic glucoside (*9*).

The detection of cyanide exposure is thus of great importance and determination of cyanide in blood, erythrocytes, or plasma has been used for this purpose. However, there are different opinions concerning the material to be preferred. Some authors recommend measuring cyanide in whole blood as most appropriate (*8, 10–13*); others claim that cyanide determinations performed on whole blood give falsely high results, due to the artifactual formation of cyanide from plasma thiocyanate and oxyhemoglobin (*14*) and instead advocate the determination of cyanide in plasma. It should be noted in this context that, in whole blood, cyanide is mainly confined to the erythrocytes (*7, 10, 14, 15*) but the mechanism behind this distribution has not been elucidated. It may be due to binding of cyanide to methemoglobin in erythrocytes (*10, 13*), but no experimental evidence for this has been supplied, and this hypothesis has been refuted by others (*16*). A serious drawback to the use of plasma cyanide determination as a marker of exposure to cyanide is the pronounced instability of cyanide in plasma (*11, 12*), requiring rapid analysis immediately after the sample is collected. Also, cyanide appears to be unstable in whole blood, although the evidence for this appears somewhat controversial (*11, 12, 17, 18*).

[1] Department of Clinical Chemistry, University of Linköping, S-581 85 Linköping, Sweden.
[2] Department of Paediatrics, Uppsala University, S-751 85 Uppsala, Sweden.
[3] To whom correspondence should be addressed.
Received November 26, 1984; accepted January 28, 1985.

We now report a method for determination of cyanide in whole blood, erythrocytes, and plasma, in which the cyanide is stabilized by silver ions (*19*). Hydrogen cyanide is then liberated by acidification, transferred from the sample to sodium hydroxide by aeration, and determined colorimetrically by a previously described modification of the König reaction (*20*). We also present strong evidence in favor of cyanide being bound to erythrocytes as methemoglobin cyanide.

Materials and Methods

Sulfuric acid, 11 mol/L. Add 400 mL of concentrated H_2SO_4 (relative density 1.84), in small portions and with cooling, to 300 mL of de-ionized water.

Antifoam agent. Antifoam B.

Acid silver sulfate, 20 mmol/L. Dissolve 1.56 g of Ag_2SO_4 and 25 mL of 0.5 mol/L H_2SO_4 in de-ionized water and dilute to 250 mL.

Sodium hydroxide, 0.1 mol/L. Dissolve 4.0 g of NaOH in water and dilute to 1 L.

Acetic acid, 2 mol/L. Dilute 28.6 mL of concentrated acetic acid with water to 250 mL.

Sodium hypochlorite, 5 mmol/L. Dilute 0.1 mL of NaClO, 0.5 mol/L in 0.1 mol/L NaOH (reagent no. 23039; BDH Chemicals, Poole, England), to 10 mL with de-ionized water. Prepare this solution daily.

Barbituric acid–pyridine reagent. Dissolve 6.0 g of barbituric acid in a mixture of 30 mL of pyridine and 64 mL of water, and add 6.0 mL of concentrated HCl.

Potassium cyanide stock standard solution, about 0.5 mol/L. Dissolve 3.26 g of KCN in de-ionized water and dilute to 100 mL. Standardize this solution by titration with standard $AgNO_3$ (*13*) to determine the actual CN^- concentration in the stock solution.

Potassium cyanide working standard, about 1 mmol/L. Dilute the stock KCN solution 500-fold with de-ionized water. Prepare this solution freshly as needed.

Material for Analysis

Freshly drawn heparinized blood is used after determination of its hematocrit. For analysis of whole blood, 1.0 mL was pipetted into 10.0 mL of the acid silver sulfate reagent. For analysis of erythrocytes and plasma, the blood was transferred to a centrifuge tube and the upper level of the blood was marked on the tube with a felt-tip marking pen. After centrifugation for 10 min at 1100 × *g*, the plasma was removed and isotonic saline was added to the mark. The erythrocytes were suspended in the saline by repeated inversion of the tube and 1.0 mL of this suspension was added to 10.0 mL of acid silver sulfate reagent for determination of erythrocyte cyanide. For measurement of cyanide in plasma, 4.0 mL of plasma was added to 10 mL of acid silver sulfate reagent. Samples were stored at −20 °C if not analyzed immediately.

Analysis

General method. Prepare for each sample (blood, erythrocytes, or plasma treated with acid silver sulfate) a simple

aeration apparatus (Figure 1) as follows. Connect a 150 × 25 mm side-arm tube fitted with a one-hole silicone rubber stopper carrying a glass inlet tube (7 mm o.d.) by means of a short length of rubber latex tubing to a 150 × 20 mm side-arm tube carrying a glass inlet tube (7 mm o.d.) and a one-hole silicone rubber stopper. Place the sample and five drops of antifoam agent in the larger side-arm tube and 4 mL of 0.1 mol/L NaOH in the smaller side-arm tube and add 5 mL of 11 mol/L H_2SO_4 to the sample. Transfer the liberated HCN to the NaOH trap by aeration with nitrogen (which has been purified by passage through a gas-washing bottle containing 0.1 mol/L NaOH) at a flow rate of 0.5 L/min. Up to six pairs of sample tubes and traps may be connected in series in this step. After the aeration has proceeded for 2 h, turn off the nitrogen and remove the stoppers from the NaOH traps, with care to blow any NaOH from the inlet tube back into the trap. Add 0.4 mL of 2 mol/L acetic acid, vortex-mix briefly, add 0.1 mL of 5 mmol/L NaClO, and mix again. Within 1 min, add 0.5 mL of the barbituric acid–pyridine reagent, mix, and determine the absorbance at 580 nm in a 1-cm cuvet 5 to 15 min later. A blank determination is always performed, in which 10 mL of acid silver sulfate reagent is taken as sample. Read the cyanide concentration in the sample from a standard graph prepared with cyanide standards of known concentrations. This graph is linear up to cyanide concentrations corresponding to at least 50 μmol per liter of whole blood and 12.5 μmol per liter of plasma.

Alternative method. This method was used in experiments with samples not stabilized by the addition of acid silver sulfate reagent. In this case 1.0 mL of whole blood or erythrocyte suspension or 4.0 mL of plasma was added to 10 mL of de-ionized water in the sample tube and five drops of antifoam agent were added. The sample was acidified by addition of 1.0 mL of 0.5 mol/L H_2SO_4 and the liberated HCN was then transferred by aeration into 4.0 mL of 0.1 mol/L NaOH as in the general method except for the aeration period being only 45 min. The remaining steps were then the same as in the general procedure.

Other methods. Methemoglobin was determined according to van Slyke et al. (*21*).

Results

Analytical Considerations

Procedure. The conditions for liberation of cyanide from silver cyanide were based on a procedure for the collection of hydrogen cyanide in respiratory air (*19*). We verified that the analytical recovery of cyanide added to the Ag_2SO_4 reagent was quantitative. The colorimetric method for determination of cyanide was a modification of the König reaction (Figure 2) earlier used for the determination of thiocyanate. We found that optimal reaction conditions for the conversion of cyanide to cyanogen chloride were very similar to those used earlier for the chlorination of thiocyanate with sodium hypochlorite (*20*). The reaction is thus practically instantaneous and should be performed under slightly acid conditions, with use of a concentration of hypochlorite between 0.01 to 0.1 mmol/L. The further conversion of cyanogen chloride to glutaconic aldehyde and formation of the chromogen with barbituric acid are steps identical to those involved in thiocyanate determinations, and the optimal reaction conditions are the same.

Stability of cyanide added to blood, erythrocytes, and plasma. We examined the effect of storage at different temperatures on cyanide added to blood, erythrocytes, and plasma, using both samples treated with Ag_2SO_4 and untreated samples. As shown in Figure 3, cyanide added to whole blood was stable at all temperatures studied for more than two weeks in the presence of Ag_2SO_4, whereas untreated samples were only stable at −20 °C. Similar results were obtained with erythrocytes (Figure 4). When cyanide was added to plasma (Figure 5) and analyzed within a few minutes, only 80% of the cyanide added was accounted for in

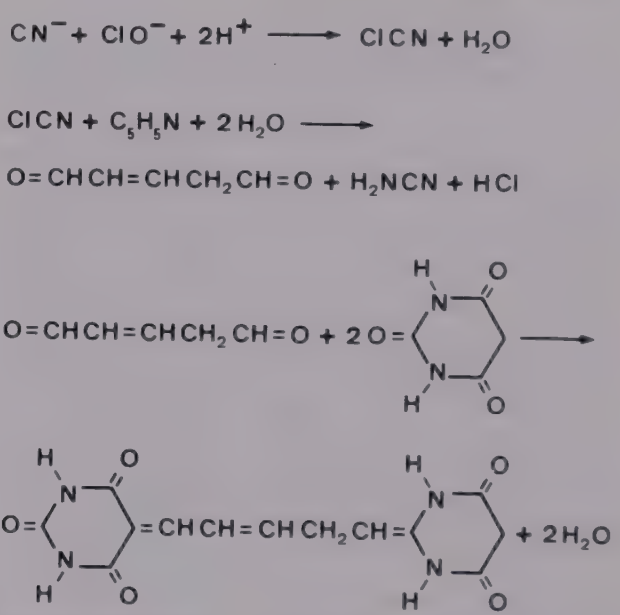


Fig. 2. The König reaction for determination of cyanide

Cyanide is first converted to cyanogen chloride by hypochlorite. Cyanogen chloride then reacts with pyridine to produce glutaconic aldehyde, which finally is coupled with barbituric acid yield to a chromogen

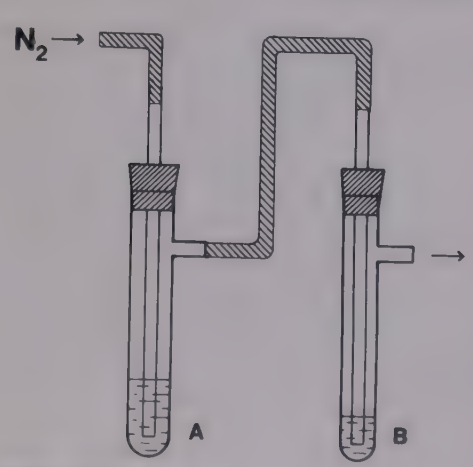


Fig. 1. Aeration apparatus

A, tube to contain sample; *B*, trap to contain NaOH

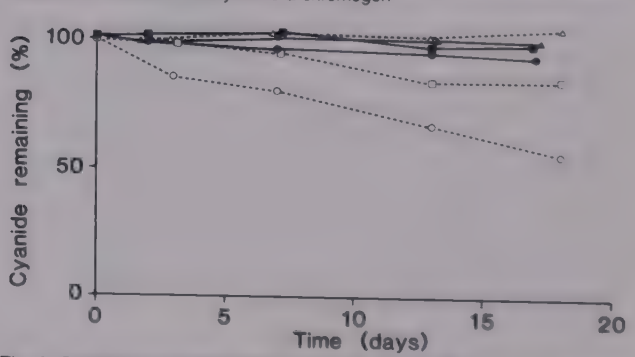


Fig. 3. Stability of cyanide added to whole blood with (*dark symbols*) or without (*open symbols*) Ag_2SO_4 at −20 °C (△,▲), 4 °C (□,■), or room temperature (○,●)

Initial cyanide concentration in samples with Ag_2SO_4 was 48 μmol/L and in samples without Ag_2SO_4 23 μmol/L

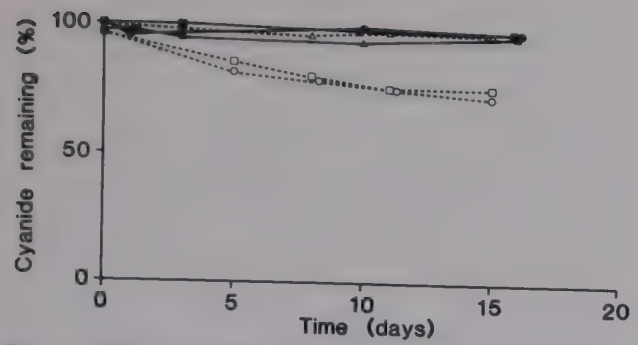


Fig. 4. Stability of cyanide added to erythrocytes with (*dark symbols*) or without (*open symbols*) Ag_2SO_4 at −20 °C (△,▲), 4 °C (□,■), or room temperature (○,●)

Initial cyanide concentration 29 μmol/L

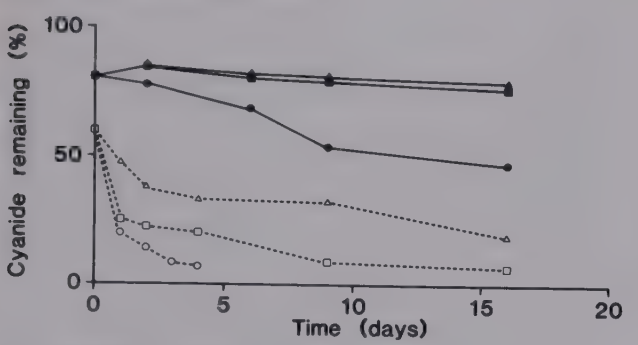


Fig. 5. Stability of cyanide added to plasma with (*dark symbols*) or without (*open symbols*) Ag_2SO_4 at −20 °C (△,▲), 4 °C (□,■), or room temperature (○,●)

Initial cyanide concentration 16 μmol/L

samples treated with Ag_2SO_4, but the remaining cyanide was reasonably stable in the freezer (−20 °C) and in the refrigerator (−4 °C) although not at room temperature (23 °C). From plasma samples not treated with Ag_2SO_4 only 60% of added cyanide could be reduced initially and cyanide then rapidly disappeared at all temperatures studied (Figure 5).

Disappearance of measurable cyanide from plasma and solutions of serum albumin. The results from the stability studies indicated that cyanide added to plasma was rapidly transformed to a form not measurable with the present method. Reportedly, cyanide reacts with serum albumin but no kinetic data were presented (*16, 22*). Consequently the disappearance of cyanide from plasma and from a solution containing the same concentration of serum albumin was then studied. The results we obtained (Figure 6) strongly suggest that the rapid disappearance of measurable cyanide from plasma is attributable to a reaction with serum albumin.

Partition of cyanide between erythrocytes and plasma. When we added increasing amounts of cyanide to whole blood, most of the cyanide was initially found in the erythrocytes and very little was detected in plasma (Figure 7). Suddenly, however, the concentration in plasma markedly increased and that in erythrocytes leveled off, and when we plotted the cyanide concentrations in erythrocytes and plasma vs the amount of cyanide added, we obtained typical titration curves (Figure 7). From the inflection points of these curves it could be calculated that the saturable component in erythrocytes bound 89 μmol of cyanide per liter of blood. The methemoglobin concentration of this blood sample was found to be 0.68% of total hemoglobin, corresponding to a ferriheme concentration of 65 μmol/L. A similar experiment on another blood sample gave a cyanide-binding capacity of 87 μmol/L blood and a methemoglobin

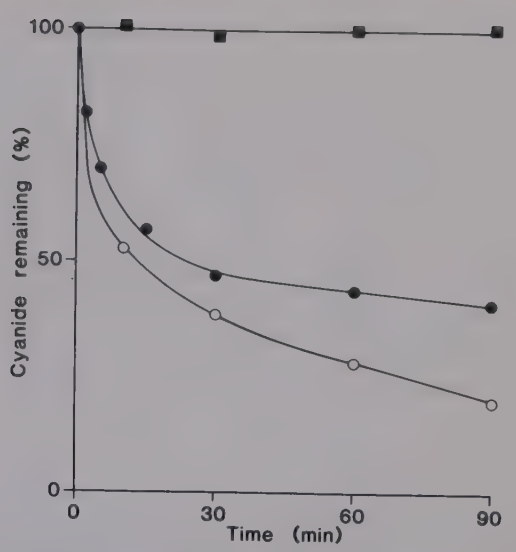


Fig. 6. Analytical recovery of cyanide added to plasma (●), serum albumin solution (○) or buffer (■)

The concentration of albumin in the plasma sample was 42.2 g/L and the albumin solution contained, per liter, 40.0 g of human serum albumin (4× crystallized; ICN Pharmaceuticals, Cleveland, OH) in a mixture containing 25 mmol of Na_2HPO_4 and 100 mmol of NaCl per liter, final pH 7.5. Initial cyanide concentration, 20 μmol/L

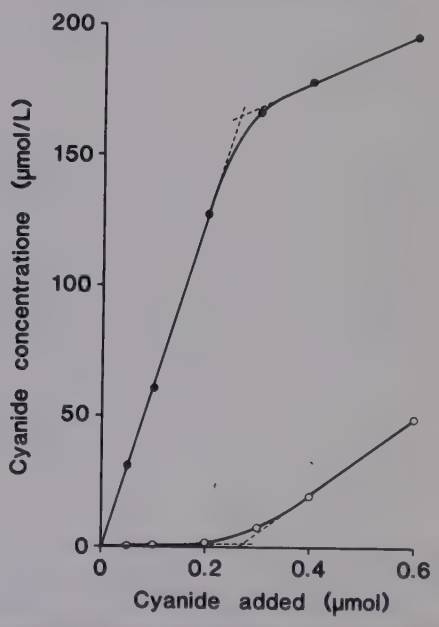


Fig. 7. Cyanide concentration in plasma (○) and erythrocytes (●) as a function of cyanide added to whole blood (3 mL)

concentration equivalent to a ferriheme concentration of 58 μmol/L blood. Furthermore, by addition of sodium nitrite (*23*) the methemoglobin content of a blood sample was artifactually increased to a concentration equivalent with 479 μmol ferriheme/L. The cyanide-binding capacity of this blood sample was 517 μmol/L, in satisfactory agreement with its ferriheme concentration. Thus, our results suggest that most of the cyanide-binding capacity of normal erythrocytes is due to methemoglobin.

Sensitivity, precision, and recovery. The sensitivity of the method, defined as the concentration corresponding to twice the SD of blank determinations, was 0.15 nmol per assay, corresponding to a concentration of 0.15 μmol/L when whole blood or erythrocytes were analyzed, and to 0.04 μmol/L when plasma was analyzed.

The intra-assay precision of the method was evaluated by analysis of five replicate samples supplemented with cya-

nide to give a concentration of 10 μmol/L in whole blood and erythrocytes, 2.5 μmol/L in plasma. The mean (± 1 SD) result for whole blood was 9.64 ± 0.16 μmol/L, corresponding to a yield of 96.4% and a CV of 1.6%. For erythrocytes the cyanide concentration found was 9.68 ± 0.12 μmol/L, corresponding to a yield of 96.8% and a CV of 1.2%. For plasma the measured cyanide concentration was 1.69 ± 0.06 μmol/L, equivalent to a yield of only 67.6% and a CV of 3.6%.

The inter-assay precision was evaluated from results obtained on samples stored at −20 °C, assuming that the cyanide was stable under these conditions (*cf.* Figure 3–5). For whole blood a mean (± SD) value of 48.1 ± 0.6 μmol/L (n = 5) was obtained, corresponding to a CV of 1.2%; for erythrocytes the value was 25.9 ± 0.5, corresponding to a CV of 1.9%, and for plasma this value was 13.8 ± 0.4, corresponding to a CV of 2.9%.

Interference. Acetonitrile and amygdaline (10 μmol/L added to 1 mL of plasma) gave no detectable cyanide in the assay. On the other hand, sodium nitroprusside caused a positive interference. When 50 nmol of nitroprusside was added as an aqueous solution, 1.4 nmol cyanide was determined, but when the same amount of nitroprusside was added to 1 mL of plasma, 83.7 nmol of cyanide was obtained, corresponding to 33% of the cyanide available in nitroprusside. The mechanism behind this effect is unknown. When 50 nmol of nitroprusside added to 1 mL of plasma was analyzed by the alternative method only 0.7 nmol of cyanide was obtained, and this method should thus be used for the assay of cyanide in samples from patients treated with nitroprusside.

Because it has been claimed (*14*) that the thiocyanate present in plasma can result in falsely high cyanide concentrations in whole blood when the latter is taken directly for analysis, we performed the following experiments. Two samples of human blood, analyzed for cyanide, gave 0.09 and 0.11 μmol/L. Thiocyanate was also determined in plasma from these blood samples, and values of 35 and 41 μmol/L were obtained. The blood samples were then supplemented with thiocyanate to give a thiocyanate concentration of 400 μmol/L in the plasma, and the whole-blood cyanide was again determined. The results obtained, 0.09 and 0.11 μmol/L, demonstrated that no interference was caused by thiocyanate.

Values for Normal Subjects

The method described was used to measure the cyanide concentration in whole blood, erythrocytes, and plasma from 10 non-smoking subjects (two men and eight women). The mean results (± SD) obtained were 0.13 ± 0.08 μmol/L for whole blood, 0.24 ± 0.22 μmol/L for erythrocytes, and 0.02 ± 0.02 μmol/L for plasma. Corresponding values for five smoking subjects (one man, four women, each of whom had smoked a cigarette within 1 h before blood sampling) were 0.33 ± 0.12 μmol/L for whole blood, 0.68 ± 0.20 μmol/L for erythrocytes, and 0.03 ± 0.02 μmol/L for plasma. The values for whole blood and erythrocytes from smoking subjects were significantly higher than those of non-smokers, whereas the values for plasma were not significantly different in the two groups. In fact, the plasma values for plasma and that for whole blood in non-smokers were below the detection limit for the method as previously defined.

Discussion

We find cyanide in whole blood to be reasonably stable in the absence of silver ions at −20 °C, whereas there are losses at higher temperatures. These results are at variance with some earlier studies. Pettigrew and Fell (*12*) thus found that cyanide added to whole blood was stable at both 4 °C and −20 °C for several days, whereas Ballantyne (*17*) reported that cyanide markedly increased when whole blood was stored at −20 °C, but was more stable at 4 °C or room temperature. On the other hand, Egekeze and Oehme (*18*) found the opposite: a more pronounced increase of blood cyanide at 25 °C than at 4 °C. We can offer no explanation for these discrepanices. More important, we found whole-blood cyanide to be completely stable in the presence of silver ions at all temperatures studied. We also observed that cyanide present in erythrocytes was stable in the absence of silver ions at −20 °C but not at higher temperatures, whereas Vesey and Wilson (*14*) claimed that erythrocyte cyanide was stable for as long as a week at both 4 °C and −20 °C. Nevertheless, our results demonstrate a stabilizing effect of silver ions also on erythrocyte cyanide. This stabilizing effect is much more important when plasma is analyzed. We thus observed a rapid decrease in cyanide added to plasma, in agreement with earlier reports (*10–12*), and we also present evidence that the mechanism for this is a reaction with serum albumin. Catsimpoolas and Wood (*22*) studied the reaction between bovine serum albumin and cyanide and found that, at neutral pH, cystine residues in the albumin are cleaved by cyanide with formation of iminothiazolidine and cysteine residues. Our results demonstrate that this reaction is rapidly quenched by silver ions. Consequently, plasma samples should be treated with the acid silver sulfate reagent as promptly as possible after collection of blood samples if results for plasma cyanide determinations are to be meaningful. The samples may then be stored in the refrigerator for at least two weeks before analysis. The stabilizing effect of silver ions on cyanide in biological fluids is of special importance for studies of cyanide levels in cassava-eaters. Populations exposed to the high concentrations of cyanide in cassava live in remote rural areas. The instability of cyanide has up to now precluded the transport of specimens to distantly located laboratories for later analysis, but this obstacle may be circumvented by use of the present method.

Most of the cyanide added to whole blood is trapped in the erythrocytes (*10, 14, 15*), and we have now demonstrated that this is due to a saturable component. Although it has been suggested (*10, 13*) that cyanide in erythrocytes is bound to methemoglobin, no experimental evidence for this proposal has been presented. We have now determined the concentration of the saturable cyanide-binding component in erythrocytes and also determined their methemoglobin content. It should be noted in this context that measurements of methemoglobin at the low concentrations present in normal blood are fairly imprecise, but the values we obtained were in good agreement with those reported by others (*21, 24*). The results obtained strongly suggest that most of the cyanide-binding capacity of normal erythrocytes is attributable to methemoglobin. On the other hand, the proposal by McMillan and Svoboda (*16*) that cyanide in erythrocytes is bound to hemoglobin is obviously untenable, as the affinity of hemoglobin for cyanide is very low (*25*) in contrast to that of methemoglobin (*26*). Furthermore, a saturation of cyanide binding to hemoglobin should not be observed at cyanide concentrations below 8 mmol/L blood (the concentration of ferroheme present as hemoglobin in normal blood). However, we found a saturation effect of cyanide binding at fer lower concentrations of cyanide, equivalent to the methemoglobin content of the blood.

The sensitivity of the present method is adequate for the determination of cyanide in blood, erythrocytes, or plasma of persons heavily exposed to cyanide (*27*). We advocate the

determination of cyanide in whole blood as the initial analysis for assessment of cyanide exposure. However, increased values of plasma cyanide indicate a very serious exposure to cyanide and determinations of cyanide in plasma may thus be a valuable adjunct in such circumstances. On the other hand, the use of values for cyanide in blood, erythrocytes, or plasma as an indicator of tobacco smoking appears more controversial. It was thus claimed (*12*) that there is no significant difference between whole-blood cyanide in non-smokers and smokers, whereas others (*3, 17*) found increased cyanide in blood from smokers, which we confirm in the present investigation. We furthermore confirm an earlier observation (*28*) that there is no significant difference between plasma cyanide concentrations in smokers and non-smokers. It should be noted in this context that values reported in the literature for whole-blood cyanide in non-smokers range from 0.62 ± 0.24 μmol/L (*17*) to 2.9 ± 2.4 μmol/L (*3*). We obtained an even lower value (0.13 ± 0.08 μmol/L), which was in fact below the true detection limit of the method. These discrepancies are probably explained by different sensitivities of the methods used and true values for blood cyanide in non-smokers are probably not obtainable by present methods. Although we found a significantly higher value for blood cyanide in smokers, we believe that more sensitive analytical methods are required for the use of whole-blood cyanide as an indicator of tobacco smoking.

This work was supported by grant No. 03X-05644 from the Swedish Medical Research Council and by grant no. 83/204 from the Swedish Agency for Research Cooperation with Developing Countries.

References

1. Bark LS, Higson HG. A review of the methods available for the detection and determination of small amounts of cyanide. *Analyst* **88**, 751–769 (1963).

2. Labianca DA. On the nature of cyanide poisoning. *J Chem Educ* **56**, 788–791 (1979).

3. Symington IS, Andersson RA, Thomson I, et al. Cyanide exposure in fires. *Lancet* **ii**, 91–92 (1978).

4. Brunnemann KD, Yu L, Hoffman D. Chemical studies on tobacco smoke. XLIX. Gas chromatographic determination of hydrogen cyanide and cyanogen in tobacco smoke. *J Anal Toxicol* **1**, 38–42 (1977).

5. Wilson J. Cyanide in human disease. A review of clinical and laboratory evidence. *Fundam Appl Toxicol* **3**, 397–399 (1983).

6. Winek CL, Fusia E, Collom WD, Shanor SP. Cyanide poisoning as a mode of suicide. *Forensic Sci* **11**, 51–55 (1978).

7. Vesey CJ, Cole PV, Simpson PJ. Cyanide and thiocyanate concentrations following sodium nitroprusside infusion in man. *Br J Anaesth* **48**, 651–660 (1976),

8. Bogusz M, Moroz J, Kanski J, et al. Blood cyanide and thiocyanate concentrations after administration of sodium nitroprusside as hypotensive agent in neurosurgery. *Clin Chem* **25**, 60–63 (1979).

9. Cock JH. Cassava: A basic energy source in the tropics. *Science* **218**, 755–762 (1982).

10. Baar S. The micro determination of cyanide: Its application to the analysis of whole blood. *Analyst* **91**, 268–272 (1966).

11. Ballantyne B, Bright J, Williams P. An experimental assessment of decreases in measurable cyanide levels in biological fluids. *J Forensic Sci Soc* **13**, 111–117 (1973).

12. Pettigrew AR, Fell GS. Microdiffusion method for estimation of cyanide in whole blood and its application to the study of conversion of cyanide to thiocyanate. *Clin Chem* **19**, 466–471 (1973).

13. Rodkey FL, Collison HA. Determination of cyanide and nitroprusside in blood and plasma. *Clin Chem* **23**, 1969–1975 (1977).

14. Vesey CJ, Wilson J. Red cell cyanide. *J Pharm Pharmacol* **30**, 20–26 (1978).

15. Sunshine I, Finkle B. The necessity for tissue studies in fatal cyanide poisoning. *Int Arch Gewerbepath Gewerbehyg* **20**, 558–561 (1964).

16. McMillan DE, Svoboda AC. The role of erythrocytes in cyanide detoxification. *J Pharmacol Exp Ther* **221**, 37–42 (1982).

17. Ballantyne B. In vitro production of cyanide in normal human blood and the influence of thiocyanate and storage temperature. *Clin Toxicol* **11**, 173–193 (1977).

18. Egekeze JO, Oehme FW. Direct potentiometric method for the determination of cyanide in biological materials. *J Anal Toxicol* **3**, 119–124 (1979).

19. Boxer GE, Richards JC. Determination of traces of hydrogen cyanide in respiratory air. *Arch Biochem Biophys* **39**, 287–291 (1952).

20. Lundquist P, Mårtensson J, Sörbo B, Öhman S. Method for determining thiocyanate in serum and urine. *Clin Chem* **25**, 678–681 (1979).

21. Van Slyke DD, Hiller A, Weisiger JR, Gruz WD. Determination of carbon monoxide in blood and of total active hemoglobin by carbon monoxide capacity. Inactive hemoglobin and methemoglobin contents of normal human blood. *J Biol Chem* **166**, 121–148 (1946).

22. Catsimpoolas N, Wood JL. The reaction of cyanide with bovine serum albumin. *J Biol Chem* **239**, 4132–4137 (1964).

23. Greenberg LA, Lester D, Haggard HW. The reaction of hemoglobin with nitrite. *J Biol Chem* **151**, 665–673 (1943).

24. Leaky T, Smith R. Notes on methemoglobin determination. *Clin Chem* **6**, 148–152 (1960).

25. Stitt F, Coryell CD. Magnetic study of the equilibrium between ferrohemoglobin, cyanide ion and cyanide ferrohemoglobin. *J Am Chem Soc* **61**, 1263–1266 (1939).

26. Coryell CD, Stitt F, Pauling L. The magnetic properties and structure of ferrihemoglobin (methemoglobin) and some of its compounds. *J Am Chem Soc* **59**, 633–642 (1937).

27. Ansell M, Lewis FAS. A review of cyanide concentrations found in human organs. A survey of literature concerning cyanide metabolism, "normal," non-fatal, and fatal body cyanide levels. *J Forensic Med* **17**, 148–155 (1970).

28. Wilson J, Matthews DM. Metabolic inter-relationships between cyanide, thiocyanate and vitamin B_{12} in smokers and non-smokers. *Clin Sci* **31**, 1–7 (1966).

ANKLE CLONUS CORRELATING WITH CYANIDE INTAKE FROM CASSAVA IN RURAL CHILDREN FROM MOZAMBIQUE.

Julie Cliff(1), Sander Essers(2) and Hans Rosling(3)

(1) Epidemiological section, Ministry of Health, Mozambique.

(2) Provincial Directorate of Health, Nampula, Mozambique.

(3) Department of Internal Medicine and International Child Health Unit, University Hospital, Uppsala, Sweden.

Correspondence should be addressed to:
Hans Rosling, International Child Health Unit, University Hospital, S-751 85 Uppsala, Sweden.

ABSTRACT

Dietary cyanide intake measured as urinary thiocyanate excretion and neurologic function were studied in apparently healthy schoolchildren from an area in Mozambique previously affected by an epidemic of spastic paraparesis attributed to cyanide exposure from cassava. Cassava consumption, cyanide exposure and ankle clonus rate were all higher in these children than in children from neighbouring areas where no paraparesis had been reported.
Furthermore, thiocyanate excretions were higher in children with clonus compared to children without clonus from the same area. These results support the hypothesis that cyanide exposure under certain conditions can cause lesions of the cortico-spinal tracts. The rate of ankle clonus in school children may serve as an epidemiological indicator of this disorder.

KEY WORDS: cassava, cyanide, thiocyanate, spastic paraparesis, ankle clonus

INTRODUCTION

Due to its high yields, cassava is an important staple crop in the tropics(1). A problem connected with cassava is its content of cyanogenic glucosides. If these are not removed before consumption, a high cyanide intake may occur, and this has been suggested as an etiological factor in certain types of nutritional neuropathies(2,3).
A recently reported epidemic of spastic paraparesis that mainly affected children in a drought-stricken area of Mozambique was also attributed to a high cyanide exposure from cassava(4) in combination with other dietary deficiencies (5,6). The epidemic occurred during the cassava harvest period in 1981 when the population, due to food shortage, had to consume toxic varieties of cassava without the necessary

processing for removal of cyanide. A high intake of cyanide was verified by increased serum levels of thiocyanate, the main metabolite of cyanide.

As could be expected after a severe drought, the availability of food improved only slowly, and during the following cassava harvest periods the population still had to consume roots with a high cyanide content. New outbreaks of spastic paraparesis could thus be expected to occur in the same season of the following years, as has been reported from cassava-growing populations in Zaire(7,8).

However, surveillance in the years following the drought only revealed sporadic cases of paraparesis. On the other hand a physiotherapist reported that ankle clonus was common among apparently healthy children in the area. The latter observation was of interest as the spastic paraparesis observed was consistent with a myelopathy (9) affecting the cortico-spinal tracts, and a sustained ankle clonus was a very prominent symptom of the disease(4). Clonus is caused by stretch reflexes which are repeatedly elicited due to a reduced pre-synaptic inhibition of the reflex arch(10) due to lesions to the cortico-spinal tracts. The finding of clonus in an apparently healthy child may indicate a minor lesions to these tracts and it was thus possible that surveillance of the ankle clonus rate could be used for monitoring the risk of a new epidemic of spastic paraparesis.

We therefore examined school children from the area that had previously been affected by spastic paraparesis as well as from areas where the disease had not occurred. The aim was to study whether the ankle clonus rate, on the one hand correlated with the prevalence of the disease, and, on the other hand, if it correlated with current cyanide exposure from cassava, measured as the urinary thiocyanate excretion.

MATERIAL AND METHODS

Area

The study was carried out in the Memba district, Mozambique, which is inhabited mainly by peasants growing cassava, maize, sorghum and beans. In 1981 this district suffered from a severe drought in which toxic varieties of cassava were the only crops to survive. The roots are normaly sun-dried and stored for two months to reduce the cyanide content before consumption, but due to lack of other food after the drought, the roots were consumed within some days of harvesting(5). The present study was done 2 years after the drought when the dietary situation still was unsatisfactory and cassava roots were consumed after about one month of sun-drying.

Subjects

Neurological examinations were performed on 189 children from two schools in the rural area previously affected by paraparesis. Furthermore, 134 school children from the semiurban administrative centre of the Memba district and 52 urban children from Nampula city, 300 km away, were examined as controls. No cases of paraparesis had been found in the semiurban and urban populations. All children were in the first year of school, the proportions of girls in the three groups were 45%, 51% and 44% respectively and the mean age($\pm$SEM) were 8.9$\pm$0.1, 8.9$\pm$0.2 and 7.0$\pm$0.1 years respectively.

Nutritional information and urine specimens were obtained from the first 32 and 31 children, respectively, in the two rural schools and from the first 30 and 28 children, respectively, from the other two areas. The mean($\pm$SEM) age of the urine tested children from the three different areas was 9.4$\pm$0.2, 9.3$\pm$0.3 and 7.1$\pm$0.1 years respectively.

Methods

After the gait had been observed, the knee-jerk was tested with the child sitting on a table. Ankle clonus was tested by

sharply dorsiflexing the ankle when the child was sitting with the lower part of the legs hanging relaxed over the edge of the table. A response in at least one leg of three or more rhythmical contractions with the typical frequency of about 5 Hz(10), was considered as positive for clonus, and this was found easy to standardize between the two examiners.

The mean number of meals per child per day that contained cassava and cereals, respectively, was calculated based on a 24-hour recall of all food items consumed by each child.

The day was divided into three periods and all food items consumed were refered to one of these periods so that three meals per day became a maximum (11). A double-check with available older family members revealed some under-reporting of cassava-consumption, especially in the semiurban group.

Thiocyanate was determined according to Lundquist et al.(12) using Amberlyst A21 as adsorbent(13). As 24-hour urine collections could not be performed the thiocyanate excretion was determined as mmol/mol of creatinine, the latter determined by a routine alkaline picrate method. The mean ($\pm$SEM) creatinine levels were similar in the three groups studied, 9.7$\pm$0.6, 8.9$\pm$1.2 and 7.5$\pm$0.8 mmol/l in the rural, semiurban and urban group, respectively.

RESULTS

Table 1 shows that the proportion of children with clonus was higher in the area affected by spastic paraparesis than in the non-affected areas. Exaggerated reflexes were common in the area affected by the epidemic as well as in the non-affected semiurban area but less common in the urban area. In all areas exaggerated reflexes were more common than clonus: in fact they were exaggerated in 55 out of the 58 children with clonus. All but three of these 58 subjects had bilateral clonus. The proportion of children with diminished reflexes was low in all areas studied, and when observing the children walking we found no cases with gait abnormalities.

TABLE 1
The proportion of children with abnormal neurological signs in one area affected and two areas not affected by spastic paraparesis

	Affected rural-area (n=189)	Non-affected semiurban-area (n=133)	Non-affected urban area (n=52)
Clonus elicitible	41 = 22%	14 = 10% *	3 = 6% *
Knee-jerk exaggerated	91 = 48%	64 = 48%	13 = 22% *
Knee-jerk diminished	10 = 5%	9 = 7%	4 = 8%

* Significantly different from rural children by chi-square test($p < 0.05$).

Table 2 shows that cassava was the dominating staple food in the rural area. It was also an important staple food for the semiurban children but was hardly at all consumed by the urban group. The dietary cyanide exposure, as measured by the thiocyanate excretion, correlated with cassava consumption and was highest in the rural children, somewhat lower in the semiurban and very low in the urban children.

TABLE 2
Staple food consumption and urinary thiocyanate excretion in children from one area affected and two areas non-affected by spastic paraparesis.

	Affected rural area (n=63)	Non-affected semiurban area (n=30)	Non-affected urban area (n=28)
Staple food consumption:			
Meals/day with cassava	2.0	0.5	0.1
Meals/day with cereals	0.5	1.4	2.7
Thiocyanate:			
mmol/mol creatinine	80.1±6.4	52.0±12.6 *	8.9±2.7 *

The results for thiocyanate are mean±SEM.
* Significantly different from rural group by students t-test($p<0.05$).

Figure 1 shows that the 12 rural children with clonus had been moore heavily exposed to cyanide, measured as thiocyanate excretion, compared to their 51 classmates without clonus. Children without clonus had a mean (±SEM) urinary thiocyanate excretion of 70±6 mmol/mol of creatinine whereas the children with clonus had 125±13 mmol/mol of creatinine. Tested by Student's t-test, this difference was highly significant ($p<0.001$). Both the age and the urinary creatinine levels were similar in these two groups.

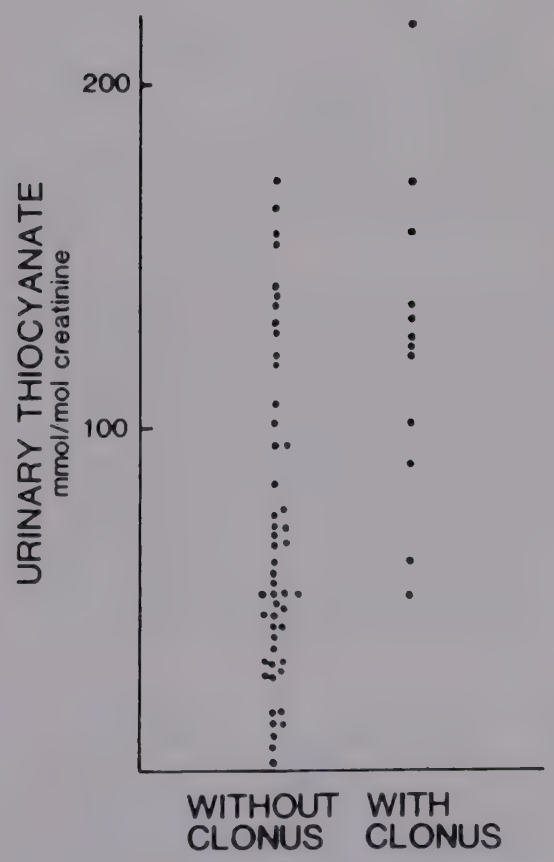


FIG. 1. Urinary thiocyanate excretion in cassava-eating rural children without and with ankle clonus.

DISCUSSION

Cassava consumption, cyanide exposure measured as urinary thiocyanate excretion and ankle clonus rate were all higher in the population that had suffered the epidemic of paraparesis than in non-affected populations. This supports the hypothesis(4) that cyanide exposure from cassava was an etiological factor of the spastic paraparesis that had occurred during the same season two years earlier when the dietary abnormalities were of a similar character but more severe.

The increased ankle clonus rate in apparently healthy children from the affected population can be explained in different ways. Firstly the children with clonus could have suffered slight but permanent damage to the cortico-spinal tracts during the epidemic two years earlier. Secondly, the ankle clonus could be a sign of a minor neurological dysfunction, reversible or permanent, acquired later but possibly by the same mechanism.

In this context it should be noted that the thiocyanate excretion found in the rural population was higher than the thiocyanate levels reported from populations with sub-acute forms of neuropathies attributed to cyanide exposure from cassava(3). This indicates that cyanide intake was still high two years after the drought and the neurological dysfunction causing an increased clonus rate could therefore possibly have been of recent origin.

That the cyanide intake obsereved in the study may cause minor neurological lesions is furthermore supported by the finding of twice as high urinary excretion of thiocyanate in the children with clonus compared to those without this sign of neurological dysfunction (Figure 1).

It must be remembered in this context that the biochemical mechanisms causing the neurological lesions attributed to cyanide exposure are poorly understood (14) and may depend on a combination of dietary deficiencies (3, 6). This is also the case with lathyrism (15), a spastic paraparesis caused by high

consumption of the drought resistant pea lathyrus sativa. This disease has several similarities to the spastic paraparesis described from Mozambique and it is of special interest in connection to this study that examination of ankle clonus in apparently healthy subjects has been of value in studies on lathyrism. Subjects with ankle clonus in presence of normal gait were found to be common in areas affected by lathyrism and these subjects were classified as cases of "latent lathyrism"(16).

When testing for clonus it must be remembered that the sign can occur physiologically(17). Clonus is easier to elicit if muscles are not relaxed and the clonic movment of the patient can start a similar reaction in the arm of the examiner, whereby results also can depend on the excitability of the examiner's own reflexes(10).

The present study show that clonus, in spite of these limitations, is a useful clinical sign if the examination procedures are standardized and the classification of responses are well defined. An examination for clonus is readily accepted by the child and is therefore rapid to perform. Ankle clonus in apparently healthy schoolchildren may therefor be a usefull indicator of minor lesions to the cortico-spinal tracts, especially in field studies on rural populations threatened by spastic paraparesis.

ACKNOWLEDGEMENTS

We thank the Ministry of Health and local health authorities in Mozambique for generous help. We also thank Ms Elena Medi, Nampula Hospital, Professor Yngve Hofvander, International Child Health Unit, Uppsala University, Sweden and Professor Bo Sörbo and Mr Per Lundquist, Department of Clinical Chemistry, Linköping University, Sweden for assistance and advice. The study was supported by grant 83/204 from the Swedish Agency for Research Cooperation with Developing Countries.

REFERENCES

1. Cutting WAM. Cassava - a valuable food but a possible poison. Tropical Doctor 1978; 8: 102-03.
2. Cruickshank EK. Effects of malnutriton on the central nervous system and the nerves. In: Handbook of clinical neurology. Vol. 28. Metabolic and deficiency diseases of the nervous system, Part 2. Amsterdam, Elsevier North Holland 1976: 1-41.
3. Osuntokun BO. Cassava diet, chronic cyanide intoxication and neuropathy in the Nigerian Africans. World Rev Nutr Diet 1981; 36: 141-73.
4. Ministry of Health, Mozambique. Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area in Mozambique. 1. Epidemiology and clinical and laboratory findings in patients. Bull WHO 1984; 62: 477-84.
5. Ministry of Health, Mozambique. Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area in Mozambique. 2. Nutritional factors and hydrocyanic acid content of cassava products. Bull WHO 1984; 62: 485-92.
6. Cliff J, Lundquist P, Mårtensson J, Rosling H, Sörbo B. Association of high cyanide and low sulphur intake in cassava-induced spastic paraparesis. Lancet (in press).
7. Anonymous. Surveillance of periferal neuropathies. Wkly Epidem Rec 1982; 57: 213-14.
8. Nkamany K, Kayinge K. Report on mission on spastic paralysis in the valley of Rivers Lukula, Luie and Inzia in Bandundu. CEPLANUT Report, Dept of Public Health, Zaire 1982; No 30.
9. Thomas PK, Schaumburg HH, Spencer PS et al. Central distal axonopathy syndromes: Newly recognized models of naturally occurring human degenerative disease. Annal Neurol 1984; 15: 313-15.
10. Hagbarth KE. Muscle spindle discharge patterns in tremor and clonus. In: Findley LJ, Capildeo F, eds. Movments disorders: Tremor. The Macmillan Press, 1984: 157-64.
11. Anonoymous. Rapid village nutrition survey technique. WHO AFR/NUTR/84, 1977.
12. Lundquist P, Mårtensson J, Sörbo B, Öhman S. Method for determining thiocyanate in serum and urine. Clin Chem 1979; 25: 678-81.
13. Lundquist P, Mårtensson J, Sörbo B, Öhman S. Adsorption of thiocyanate by anion-exchange resins and its analytical application. Clin Chem 1983; 26: 1178-81.
14. Editorial. Epidemic spastic paraparesis. Lancet 1984; ii: 904-05.
15. Spencer PS, Schaumburg HH. Lathyrism:A neurotoxic disease. Neurobehavioral Toxicology and Teratology 1983; 5: 625-29.
16. Dwivedi MP, Prasad BG. An epidemiological study of lathyrism in the district of Rewa, Madhya Pradesh. Ind Jour Med res 1964; 52: 81-116.
17. Gottlieb GL, Agarwal GC. Physiological clonus in man. Exp Neurol 1977; 54: 616-21.

THYROID FUNCTION IN A CASSAVA-EATING POPULATION AFFECTED BY EPIDEMIC SPASTIC PARAPARESIS

Julie Cliff(1), Per Lundqvist(2), Hans Rosling(3)
Bo Sörbo(2) and Leif Wide(4)

(1) Epidemiological Section, Ministry of Health, Mozambique.
(2) Department of Clinical Chemistry, Linköping University, Sweden.
(3) Department of Internal Medicine and International Child Health Unit, Uppsala University, Sweden.
(4) Department of Clinical Chemistry, Uppsala University, Sweden.

Correspondence should be addressed to:
Hans Rosling, International Child Health Unit,
University Hospital, S-751 85 Uppsala, Sweden.

ABSTRACT

Thyroid function was studied in a rural population in Mozambique that had been affected by an epidemic of spastic paraparesis attributed to dietary cyanide exposure from cassava. Laboratory investigation on a sample of this population demonstrated very high levels of serum and urinary thiocyanate, indicating a heavy exposure to cyanide. The urinary excretion of iodine was within normal limits, indicating an adequate intake of iodine. The serum levels of FT4I were somewhat decreased and serum FT3I, T3/T4 ratio and TSH were somewhat raised. This hormone pattern indicates an adaptation to the antithyroid effect of thiocyanate, but it does not indicate overt hypothyroidism. A follow-up study on school children was thus performed, and it also demonstrated high thiocyanate exposure, adequate intake of iodine but an absence of endemic goitre.
The results show that the thyroid gland, if iodine supply is adequate, is capable of adaptation to a heavy body burden of thiocyanate without development of overt hypothyrodism or goitre.

Key words: cassava, cyanide, thiocyanate, thyroid function, goitre

INTRODUCTION

Cassava is the staple food in many tropical countries, mainly due to high yields of this plant even on poor soils (Cock 1982). However, cassava roots contain cyanogenic glucosides, sometimes in high amounts. Different methods are used to reduce the cyanide content before consumption. If this processing is inadequantly performed, consumers are exposed to a high cyanide intake, which may cause neurological disorders (Wilson 1983).

Another harmful effect of cyanide intake is due to its conversion to thiocyanate, which is an antithyroid and goitrogenic compound (Green 1978). A high incidence of goitre in certain areas of Nigeria (Ekpechi et al. 1966) and Zaire (Bourdoux et al. 1978: Vanderpass et al. 1984) has thus been attributed to the combined effect of iodine deficiency and thiocyanate exposure resulting from a high consumption of cassava.

The present study was initiated by an epidemic of spastic paraparesis attributed to cyanide exposure from cassava in a drought-stricken area of northern Mozambique (Ministry of Health, Mozambique 1984a; 1984b). Due to shortage of food during the drought, cassava had to be consumed without adequate processing. This resulted in a high cyanide intake as verified by increased serum levels of thiocyante.

During the recovery after the drought the Ministry of Health performed an epidemiological and nutritional surveillance in the area affected by the epidemic. The present study was coordinated with this activity and aimed at evaluating the risk of thyroid dysfunction in the cassava-eating population. The serum levels of thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH) were thus determined in a sample of the population. Furthermore, thiocyanate was determined in serum and urine as an indicator of cyanide exposure (Bourdoux 1978), and the urinary iodine excretion was determined as a measure of the dietary iodine intake (Follis 1964).

The laboratory investigations indicated a slight antithyroid effect of thiocyanate and therefore a follow-up study was performed on school children to determine the goitre rate as well as the urinary excretion of iodine and thiocyanate.

MATERIALS AND METHODS

The study was carried out in the Memba District, situated in northern Mozambique and bordering on the Indian Ocean. The district is mainly inhabited by peasants growing cassava, beans, maize and sorghum. Cassava is the dominating staple food but it should be noted that dried fish, bartered with fishermen, constitute an important item of the diet. At the end of the dry season the cassava roots are harvested, sun-dried and then stored for later consumption. This procedure will slowly reduce the cyanide content, and the roots are considered suitable for consumption after a drying period of two months.

During the drought in 1981, however, the roots were consumed immediately after the harvest due to a severe food shortage. During the cassava harvests in 1982 and 1983, when this study was performed, the shortage of other foods persisted and the roots were consumed after only about two and four weeks of sun-drying, respectively.

The laboratory investigations were performed during the cassava harvest in 1982 on 27 subjects aged 5-60 years, with a median age of 28 years (9 male and 9 female adults, respectively, and 9 children of more than 4 years of age). They belonged to randomly selected households in the village "Acordo de Lusaka" located in the centre of the area affected by the epidemic of paraparesis in 1981 (Ministry of Health, Mozambique 1984b). Serum and urine specimens (the latter collected with 10% thymol in isopropanol as preservative) were frozen within 6 hours of collection and then transported to Sweden for analysis. As it was not feasible to perform 24 h urine collection, the urinary excretion of thiocyanate and iodine was related to creatinine, the latter determined with a conventional alkaline picrate method.

Thiocyanate in serum and urine was determined according to Lundqvist et al (1979) using Amberlyst A 21 as adsorbent (Lundquist et al 1983). Urinary iodine was determined by the catalytic ceric-arsenate method (Sandell et al. 1936) after

separation of interfering compounds by ion-exchange on a AG 1-X4 resin according to Sacks (1964).

The serum levels of TSH, T4 and T3 were determined by radio-immunoassay methods (Odell 1965; Wide 1983). The free T4 index (FT4I) and free T3 index (FT3I) were calculated by multiplying the values for T4 or T3 by T3-uptake values, the latter determined by a commercial reagent kit (Phadebas T3U Test, Pharmacia, Uppsala, Sweden) using Sephadex as adsorbent. Values for FT4I and FT3I were compared to those of a healthy Swedish control group (15 males and 12 females, aged 18-77 with a median age of 28 years). Values for TSH were compared to those of a Swedish control group comprising 212 non-smoking adults (128 males and 84 females, aged 19-85 with a median age of 51 years).

As the laboratory investigation indicated a slight effect of thiocyanate on the thyroid function, the prevalence of goitre was determined in 276 school children from three rural primary schools in the study area. This was carried out during the cassava harvest period in the following year. The thyroid glands were examined as described by Peréz et al (1960) and regarded as enlarged if the volume of the lateral lobes exceeded that of the terminal phalanx of the thumb of the subject examined. Due to special social circumstances blood sampling was not feasible during this survey but urine samples for thiocyanate and iodine determinations could be obtained from 89 children, equally distributed between the three schools.

RESULTS

The levels of thiocyanate in serum and urine were very high, as demonstrated in figure 1. As there were no signifiant differences between children and adults, the results from the two age groups were combined, resulting in a mean serum thiocyanate of 250±24 µmol/l (±SEM) and a mean urinary excretion of 132±28 mmol/mol creatinine. These values were

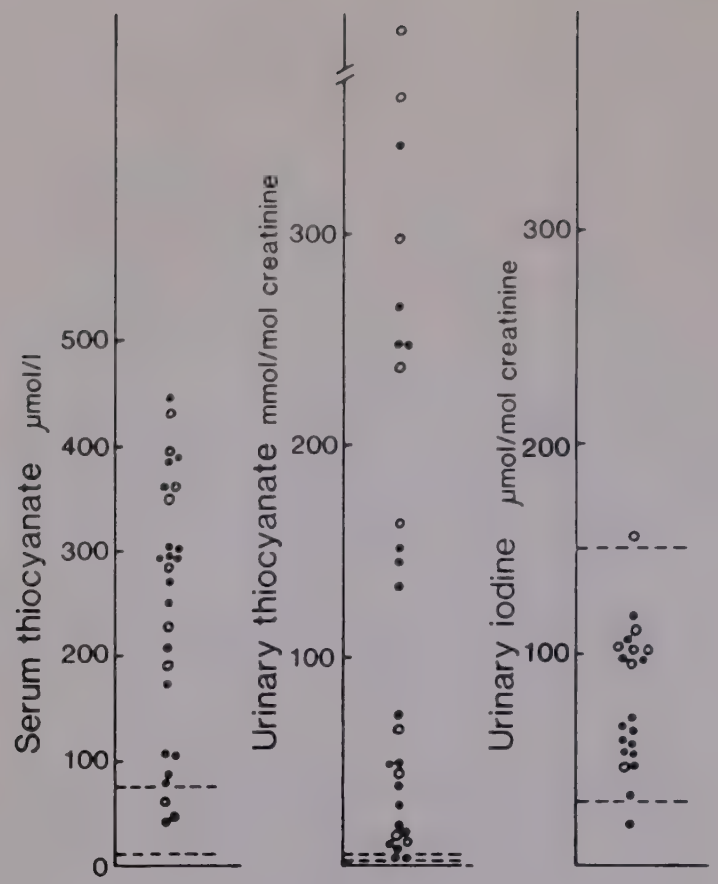


Figure 1 Serum thiocyanate, urinary excretion of thiocyanate and iodine in cassava-eating subjects. O children, ● adults and - - - reference limits of Swedish controls

respectivly 6-fold and 30-fold higher than corresponding mean values in a Swedish reference group.

Figure 1 also shows the iodine excretion, which could be determined in 21 of the subjects studied. The mean value was 79±7.3 µmol/mol creatinine and all values, except for one which was slightly raised and one slightly depressed, were within the reference limits of healthy Swedes. All but two had an iodine excretion of more than 50 µg/mg of creatinine and according to the criterion given by Follis (1964) the distribution of iodine excretion found indicates an adequate iodine intake.

Table 1 shows that TSH was increased in the cassava-consuming subjects compared to controls. FT4I was reduced compared to controls, which was mainly explained by low values among adults. FT3I on the other hand was raised compared to

Table 1

Serum levels of TSH and thyroid hormones in cassava-eating subjects compared to Swedish adults controls (mean+SEM).

Compounds	Controls	Cassava-eating subjects		
		All (n=27)	Adults (n=18)	Children (n=9)
TSH mU/l	2.1±0.09 (n=212)	4.3±0.31***	4.1±0.40***	4.7±0.49***
FT4I arb.u.	95±3.2 (n=27)	83±4.4*	74±2.9***	103±8.9
FT3I arb.u.	1.9±0.09 (n=27)	2.3±0.13*	2.0±0.14	3.0±0.13***
T3/T4 x 10	2.0±0.10 (n=27)	2.9±0.16***	2.8±0.18***	3.0±0.33***

Significant difference with Student's t-test compared to controls, * $p<0.05$ and *** $p<0.001$.

controls, mainly due to high values among children. The T3/T4 ratio, finally, was higher in the cassava-consuming group, with similar values in adults and children.

Figure 2 shows the individual values for TSH, FT4I and FT3I. All subjects had TSH values less than 10 mU/l, indicating that no one had serious hypothyroidism.

The follow-up study aiming at evaluating the goitre rate revealed only 3 subjects with enlarged thyroid glands out of the 276 children studied. This correspondes to a goitre rate of 1.1% with a 99% confidence limit of 0.1% - 4.0% , the latter calculated by using the Poisson distribution. When thiocyanate was assayed on urinary samples from school children, a mean (±SEM) value of 83.3±6.3 mmol/mol creatinine was obtained, demonstrating that their thiocyanate exposure was still high during the cassava harvest two years after the drought. The urinary iodine simultaneously determined was 222±17 µmol/mol creatinine, indicating that iodine intake was adequate and in fact higher than the previous year.

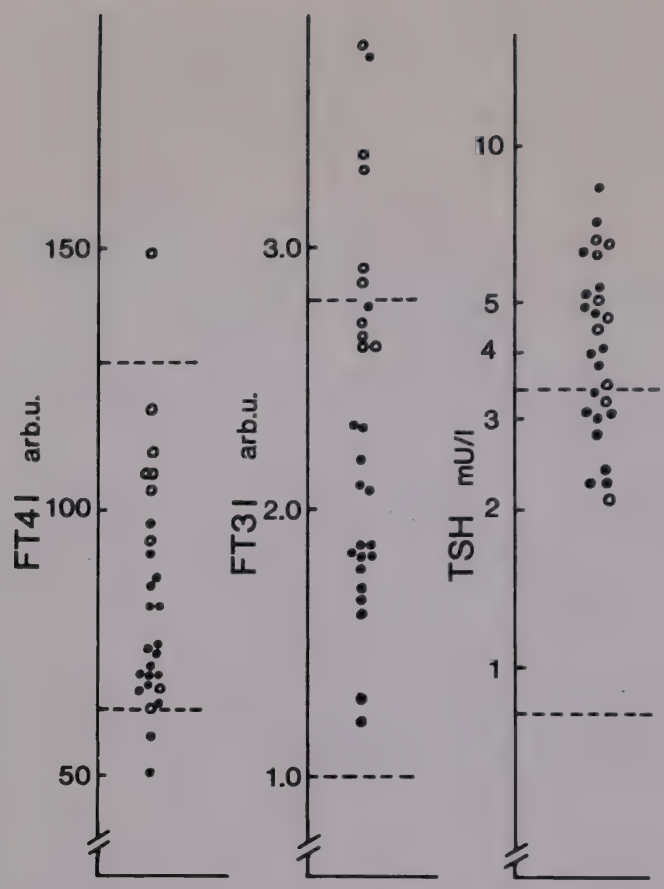


Figure 2 Serum levels of FT4I, FT3I and TSH in cassava-eating subjects. O children, ● adults and - - - reference limits of Swedish controls.

DISCUSSION

The serum and urinary levels of thiocyanate found in the present study are in fact higher than the levels reported from cassava-eating populations with endemic goitre attributed to thiocyanate exposure. Bourdoux et al. (1978) has thus found that a thiocyanate exposure resulting in a mean (±SEM) serum level of 183±1 µmol/l was related to a goitre rate of 60-70 % in a cassava-eating population from Zaire, whereas the mean serum level in the present study in Mozambique was 250+24 µmol/l. The mean urinary concentration of thiocyanate in Zaire was given as 331±10 µmol/l, and no data on creatinine concentration were presented, but the corresponding value found in the present study, 1137±187 µmol/l, was considerably higher.

Nevertheless, the frequency of goitre in the present study was only 1.1%, which is far below the arbitrary limit for endemic goitre set at 10% (Peréz et al. 1960) and corresponds to the sporadic goitre rate of populations with adequate iodine intake in industrialized countries (Thilly et al. 1980). Although it should be kept in mind that seasonal variations of cassava consumption occur in the area studied (Ministry of Health, Mozambique 1984b) and the exposure of thiocyanate might therefore not be as high throughout the year, we feel it is unlikely that the low goitre rate is explained by this mechanism.

The explanation probably lies in differences in iodine status between the populations studied in Zaire and Mozambique. The population in Zaire suffered from iodine deficiency, as demonstrated by a low urinary excretion of iodine (Bourdoux 1978) whereas the population in Mozambique had an adequate iodine intake as demonstarated by the present study. This is explained by the fact that the Mozambican population lived close to the ocean and fish was an important item of their diet. It was also found in Sicily that high exposure to thiocyanate from cabbage did not result in goitre in the presence a high iodine intake (Delange et al 1978). Delange et al.(1983) has furthermore reported a goitre rate of only 1.5 % in a cassava-eating population in Zaire with raised serum thiocyanate levels but an adequate iodine intake.

These results are easily understood if the mechanism of action of thiocyanate on the thyroid is considered. The main antithyroid effect of thiocyanate is due to its interference with the iodine accumulation of the thyroid gland (Green 1978; Ermans et al. 1980). A low dietary intake of iodine, not sufficiently low to cause goitre and thyroid insufficiency by itself may thus result in goitre if combined with exposure to thiocyanate. Similarly a heavy exposure to thiocyanate may be counteracted by a high dietary intake of iodine.

Of interest in this context is the hormone pattern found in the present study consisting of decreased FT4I and raised FT3I

as well as T3/T4 ratio and TSH. This is a typical pattern found in populations suffering from endemic goitre due to iodine deficiency (Chopra 1975), but the changes in hormone levels found in the present study were smaller than those reported from endemic goitre areas. Whereas the mean (±SEM) TSH was 31.8±3.2 mU/l in such an area in Zaire (Bourdoux 1978) and 16±2.4 mU/l in an endemic goitre area in New Guinea (Chopra et al. 1975), all values in the present study were below 10 mU/l (Figure 2).

The results of the present study thus indicate an adaptation of the thyroid function to the heavy thiocyanate exposure in the population studied and, furthermore, that the adequate iodine intake, in addition to this adaption, has protected against the development of overt hypothyroidism and goitre. In conclusion, if iodine intake is adequate, the thyroid gland is capable of adaptation to a very heavy body burden of thiocyanate from cassava without development of goitre or hypothyroidism. This has important implications for the assessment of the overall health effects of cassava-dominated diets. As cassava constitutes a staple crop with high yields and good resistance against droughts and pests, a reduced cultivation of cassava may infact impair the health of the millions of poor peasants growing this crop. On the contrary, by raising agricultural productivity an increased cultivation of cassava can improve health (Okigbo 1980), provided that efficient processing methods are used to reduce its cyanide content, that protein rich supplementary foods are available and that iodine supplementation is given when necessary.

ACKNOWLEDGEMENTS

We are indebted to the Ministry of Health, Mozambique, to Dr Anne Rodrigues, Nutritional Section of the Ministry and to Professor Yngve Hofvander, International Child Health Unit, Uppsala University, Sweden for help with the study. The study was supported by grant 83/204 from Swedish Agency for Research Cooperation with Developing Countries, by grant 03x-05644 from Swedish Medical Research Council(03X-05644) and by grants from the Maud and Birger Gustafsson Foundation.

REFERENCES

Bourdoux P, Delange F, Gerard M, Mafuta M, Hanson A & Ermans AM (1978): Evidence that cassava ingestion increases thiocyanate formation: a possible etiological factor in endemic goitre. J Clin Endocrinol Metab 46: 613-621.

Chopra I J, Hershman J M & Hornabrook R W (1975): Serum thyroid hormone and thyrotropin levels in subjects from endemic goitre regions of New Guinea. J Clin Endocrinol 40: 326-333.

Cock J H (1982): Cassava: A Basic Energy Source in the Tropics. Science 218: 755-762.

Delange F, Vigneri R, Trimarchi F, Filetti S, Pezzino V, Squatrito S, Bourdoux P & Ermans A M (1978): Etiological factors of endemic goitre in north-eastern Sicily. J Endocrinol Invest 2: 137-142.

Delange F, Bourdoux P, Colinet E et al. (1983): Nutritional Factors Involved in the Goitrogenic Action of Cassava. In: Cassava toxicity and the thyroid: Research and public health issues. Delange F & Ahluwalia R, eds. International Development Research Centre, Ottawa, Canada, IDRC-207e.

Ekpechi O L, Dimitriadou A & Fraser R (1966): Goitrogenic activity of cassava(a staple Nigerian food). Nature 210: 1137-1138.

Ermans A M, Kinthaert J, Van der Velden M & Bourdoux P (1980): Studies on the Antithyroid effects of cassava and thiocyanate in rats. In: Role of casava in the etiology of endemic goitre and cretinism. Ermans A M et al. eds. International Development Research Centre, Ottawa, Canada, IDRC-136e.

Follis R H (1964): Pattern of urinary iodine excretion in goitrous and non-goitrous areas. Amer J Clin Nutr 14: 253-268.

Green W L (1978): Mechanism of action of antithyroid compounds. In: Werner S C and Ingbar S H, eds. The Thyroid. 4th ed. New York, Hasper and Row Publishers: 48-51.

Lundquist P,Mårtensson J, Sörbo B & Öhman S (1979): Method for Determining Thiocyanate in Serum and Urine. Clin Chem 25: 678-681.

Lundquist P, Mårtensson J, Sörbo B & Öhman S (1983) Adsorption of thiocyanate by anion-exchange resin and its analytical application. Clin Chem 26: 1178-1181.

Ministry of Health, Mozambique (1984a) Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 1. Epidemiology and clinical and laboratory findings in patients. Bull WHO 62: 477-484.

Ministry of Health, Mozambique (1984b) Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 2. Nutritional factors and hydrocyanic acid content of cassava products. Bull WHO 62: 485-492.

Odell W B, Wilber J F & Paul W E (1965): Radioimmunoassay of thyrotropin in human serum. J Clin Endocrinol Metab 25: 1179-1188.

Okigbo B N (1980): The case for Cassava in the humid tropics of West Africa. Food and Nutrition Bulletin 2(4): 1-10.

Peréz C, Schrimshaw N S & Munzo J A (1960): Technique of endemic goitre surveys. In: Endemic Goitre. World Health Monograph Series 44: 369-383.

Sacks B J (1964): Recovery of Iodide from anion exchange resins. Nature 202: 899.

Sandell E B & Kolthoff I M (1937): Micro determination of iodine by a catalytic method. Mikrochem Acta 1: 9-25

Thilly C H, Delange F & Stanbury J B (1980): Epidemiological surveys in endemic goitre and cretinism. In: Endemic goitre and cretinism, iodine nutrition in health and disease. Stanbury J B & Hetzel B S eds. New York, Wiley medical publications.

Vanderpas J, Bourdoux P, Lagase R, Rivera M, Dramaix M, Lody D, Nelson G, Delange F, Ermans A M & Thilly C H (1984): Endemic infantile hypothyroidism in a severe endemic goitre area of central Africa. Clinical Endocrinology 20: 327-340.

Wide L (1981): Use of particulate immunosorbents in radioimmunoassay. Methods of Enzymolgy 73: 203-224.

Wilson J (1983): Cyanide in human disease: A review of clinical and laboratory evidence. Fundamental and Applied Toxicology 3: 397-399.

Tryckkontakt, Uppsala 1986